PERMEABILITY

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"Every protoplast is surrounded by a special dermal layer, the external plasmatic membrane or ectoplast. ... The ectoplast consists of relatively solid protoplasm, and is possessed of a special structure, by virtue of which it is able to control many of the relations which the protoplast maintains with the outer world."   G. HABERLANDT.

"There are no membranes about cells."
M. H. FISCHER.

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PERMEABILITY

CHAPTER I

INTRODUCTION

I N the study of the living organism there presents itself a well marked group of problems arising from phenomena which can be included under the term "Permeability." Every organism receives from its environment in some form or another substances which enter into its body and which either as such, or after undergoing physical and chemical change and working up into new combinations, may be carried to every part of the organism. The problems concerned in this intake into the organism of substances from the surroundings, and their passage out from the cell into the external medium, and the translocation of substances from cell to cell in the body of the organism, may be spoken of broadly as problems of permeability. It must be admitted at the outset that the word permeability in biology is largely a cloak for ignorance. When the physical chemist speaks of the permeability of a membrane, he refers to the capacity of the membrane for allowing substances to pass through it, and this is the logical and correct significance of the term. But this is not always what is meant when the physiologist speaks of the permeability of protoplasm or the permeability of an organic cell. Sometimes he means the capacity of a substance to pass into or out from the cell, sometimes its capacity to pass through one particular part of the cell, as, for instance, the protoplasm, or the surface layer of it. The cell is a complex structure, and in the present state of our analysis it is rarely possible to localise the seat of any cellular phenomena exactly, although it must be one

of the objects of research to do so. While therefore we must aim at a stricter use of the term permeability so as to conform to its usage in physical chemistry, at present our knowledge simply does not allow us to do so when we are dealing with the problems of the cell.

Research on the problems involved has proceeded along two rather distinct lines. In one, the whole living organism has been the unit of experimentation, while in the other isolated cells and tissues have been employed. In the case of unicellular and other small organisms the difference between the two groups of methods disappears. The difference between the two lines of attack is, however, very obvious in the case of work on higher plants where the methods of the first mode of attack are for the most part those of pot culture and water culture. In the hands of a number of plant physiologists from Woodward in 1699 onwards, research by these methods led to fundamentally important discoveries in regard to plant nutrition, and in the hands of Sachs and Knop about 1860 it was used to demonstrate successfully the elements essential for the nutrition and development of plants, the absorption of water and dissolved substances from the soil and the absorption of gases from, and their excretion into, the air. The methods as employed to-day have provided a quantity of empirical information on the relation between the amount of growth of plants and the constitution of the medium external to their roots; as far as permeability problems are concerned they have not led us much further than the experiments of Sachs, Knop and other workers of their time, who showed by water-culture experiments that plants were capable of absorbing certain substances through their roots, while ash analyses showed that the constituents of these substances were capable of passing through the tissues to remote parts of the organism. These methods, in short, afford no data in regard to the intake of salts by the plant and the subsequent movement of the absorbed substances, beyond the information that these take place in certain cases. Nevertheless in the past they yielded results of the first importance for our subject, and there is no reason to suppose that, with suitable modification, their period of usefulness is over.

In the animal organism similar considerations hold. From considerations based on the whole organism as a unit it early became clear that some substances could penetrate through certain cells and become absorbed into the animal, while others could not. But, generally speaking, this line of attack has not afforded quantitative

data bearing on permeability, and so has not greatly helped towards a clear insight into the problems involved.

It is to the second line of attack that we owe most of our knowledge of permeability. The use of isolated cells or isolated pieces of tissue allows the employment of more exact methods and more careful control than can always be obtained in experiments with the whole organism. Such material as roots or stems and slices of storage tissues such as tubers or fleshy roots in the case of plants, blood corpuscles, eyes, pieces of muscle in the case of animals, have formed successful objects of experimentation. The elimination of error arising from variability among different individuals, the so-called "biological error," is possible with this mode of attack, and by a proper method of experimentation results can be obtained approximating in exactness more nearly to those of physics and chemistry than is possible when whole plants or animals form the experimental object, and such results are reproducible.

As tissues differ in the form of the cells which compose them so they differ also in their functions, and it is reasonable to suppose there is no more uniformity in regard to permeability than in regard to other functions. By the use of isolated tissues we are thus on the way towards a physiological analysis which is not possible when the whole organism forms the experimental unit.

The problems of permeability are problems of general physiology; they are common to all life, plants and animals, the lowest and the highest. They are problems of the cell and of the organism as a whole. While in this account of our present knowledge of permeability we shall deal mainly with plants, we shall thus have occasionally to refer to work on animals, as this may often be helpful in understanding the conditions in plants, while the results of investigations dealing with plants from very different groups of the plant kingdom and with both whole plants and parts of plants will have to be considered.

CHAPTER II

THE SYSTEM INVOLVED

THE problem with which we are presented is then to discover the laws governing the penetration of substances into and through the living cell. It is obvious that it is of first importance to understand the system involved. Both from a chemical and physico-chemical point of view this is a very difficult matter. The essential of all living cells is protoplasm. The structure of this and its elementary properties have been described as among the most difficult problems with which the biologist has to deal (Bayliss, 1915). In its simplest form in *Amoeba* or Myxomycetes, the general body of the protoplasm, that is, the cytoplasm apart from enclosed granules, appears as a clear viscous fluid, apparently structureless, capable of changing its form under the influence of external conditions, but remaining quite distinct from, and without any tendency to mix with, the medium external to it.

It has been urged that protoplasm behaves as a liquid (Bayliss, 1915). This is shown by (1) the fact that drops of water enclosed in it assume a spherical form, (2) Brownian movement[1] observed by R. Brown in 1827 (R. Brown, 1828, 1829, 1866), (3) the action of an electric shock under the influence of which an amoeba, for instance, tends to form a sphere (Kühne, 1864), (4) the behaviour of Myxomycetes (Lister, 1888).

Under ordinary powers of the microscope certain parts of the protoplasm are visible as denser specialised organs of the cell: the nucleus, numerous small granules, and in plants the plastids. Apart from these, under the ultra-microscope (dark-ground illumination) the apparently homogeneous cytoplasm is observed not to be homogeneous, but to contain a great number of minute particles (Gaidukov, 1906–1910; Mott, 1912; Price, 1914). This is the condition characteristic of colloidal solutions, and from ultra-microscopic observation it would seem reasonable to conclude that the cytoplasm is frequently a colloidal liquid system or hydrosol,

Cytoplasm then is not homogeneous, but consists of a denser phase dispersed through the watery dispersion medium. It thus

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[1] Brownian movement of particles visible under the ordinary microscope cannot be observed in all cells or organisms (Seifriz, 1920).

has a structure, but it must be emphasized that this structure is ultra-microscopic and not coarse enough to be observed under the ordinary microscope as was at one time thought. The production of a reticulate structure in the cytoplasm when treated with fixing and staining agents led to the view that cytoplasm possessed a net structure visible under the microscope. Even if the views of some earlier writers more or less dimly suggest the opinion that cytoplasm is in the colloidal condition, and while Bütschli (1892) suggested that living protoplasm has the structure of a microscopic emulsion, it was Hardy (1899) and A. Fischer (1899) who showed that the structure of cytoplasm after treatment with fixing agents could be made to vary according to the treatment during fixation. The conclusion to be drawn is obviously that the reticulum generally observed is the result of the fixing, and for the reasons already given it must be held as undoubtedly true that cytoplasm is essentially a colloidal system.

Although in many cases the body of the cytoplasm is a sol, there is a certain amount of evidence that in many cells the cytoplasm may be in the more solid gel condition. Thus Bayliss (1919) says: "That there are possibilities of the formation of membranes, doubtless of a gel nature, within the protoplasm of a cell is shown by the fact that different reactions can take place at the same time in different parts of the cell, notwithstanding the general liquid nature of its contents." Gaidukov (1910) and Price (1914) by ultra-microscopic observation find that in some cases Brownian movement in the cell may cease, and suppose the protoplasm in these cases has taken on the state of a gel.

Price concludes that protoplasm can, and often does, exist in the gel state, and in this state may be active. Bayliss has been able to bring about the cessation of Brownian movement by weak electrical stimulation and relates the phenomenon with functional activity (1919). Chambers (1917), by microscopic observations made on dissections of living cells, also comes to the conclusion that in the ova of a number of marine organisms (*Asterias, Arbacia, Echinoarachnius, Cerebratulus, Fucus*) and in the germ cells of certain insects (*Periplaneta, Disosteira, Anasa*) as well as in protozoa, the cytoplasm usually exists as a sol. On the other hand he considers that in adult somatic cells, including nerve cells and muscle fibres, the protoplasm forms a more or less rigid gel. Leucocytes however possess a cytoplasm closely resembling that of germ cells. Seifriz 1918, 1920) also, in a similar series of observations on Myxomycetes,

oogonia, egg cells and embryos of *Fucus, Spirogyra, Vaucheria, Rhizopus* and *Zygorhynchus,* pollen tubes of *Iris versicolor, Lathyrus maritimus, Erythronium revolutum,* protozoa, and ova of *Echinoarachnius,* finds the viscosity of protoplasm varies greatly in the different cells examined. Young *Fucus* oogonia and embryos and the streaming protoplasm of Myxomycetes were the most liquid, the mature and resting eggs of marine organisms were the most viscous. This author however is of opinion (1920) that great caution should be exercised in using viscosity as the only criterion of a sol or gel condition of the protoplasm.

Price decides that in resting spores, for example, those of *Mucor,* the protoplasm is in the gel condition, but that on germination it becomes a sol. Changes in the consistency of *Fucus* eggs during maturation and fertilization are described by Seifriz (1918), while the same writer (1920) states that as a myxomycete prepares to fruit the protoplasm increases in viscosity until it becomes in consistency a gel. Seifriz has observed such changes in protoplasmic viscosity which are reversible. "The viscosity of protoplasm is not fixed, for it varies in different organisms, in the same organism at different times, and even in different regions of the same organism at the same time" (Seifriz, 1920).

Thus even where the bulk of the cytoplasm is a sol there is a considerable quantity of evidence that the surface layers of the cell plasm may be in the more solid gel condition. Loeb (1906) says confidently: "It is a general rule that every free cell is surrounded by a solid film." He instances as evidence of this the length of the pseudopodia of rhizopods, which is so great that if the pseudopodia were entirely liquid they would fall apart into droplets. As protoplasmic streaming takes place in the interior, the solid part of the pseudopodia must be at the surface. Ramsden (1894, 1903) and M. Traube (1867) have shown the formation of solid membranes at the surface of hydrosols.

In cells in which Chambers decides the cytoplasm is in the sol condition he finds the peripheral layer very dense in consistency as compared with the interior of the cell, the outer layer merging insensibly into the general body of the cytoplasm. This surface layer is particularly well marked in protozoa, for example, in *Paramoecium.* Chambers concludes that the surface layer is a "highly extensile contractile and viscous gel," which if damaged may be automatically repaired. In adult somatic cells where the interior cytoplasm is judged to be in the gel state it is not possible

to demonstrate an outer layer differing in consistency from the
rest of the cell. Price from ultra-microscopic observations states
that the presence of an outer layer of the protoplasm differentiated
from the interior seems quite definite. In any case it must be
emphasized that the surface of the cell constitutes a boundary
between two immiscible phases and as such the properties of the
surface will differ greatly from those of the main bulk of the proto-
plasm. Indeed, the surface layers of the protoplasm are generally
regarded as differing so much from the rest of the protoplasm as
to have different permeability properties. The evidence for and
against this view will be more suitably dealt with in a later chapter
when the cell membranes are considered in more detail.

The chemical composition of protoplasm is rendered difficult of
determination as the methods of chemical analysis in themselves
necessarily change the living matter into something essentially non-
living and therefore different. Chemical analysis has however pro-
vided some information regarding the components of the living sub-
stance. Water often comprises about 80 or 90 per cent. of the total
weight of protoplasm. The classical analysis of Reinke (1880) and
Reinke and Rodewald (1881) of the plasmodium of the myxomy-
cete *Fuligo varians* showed that about 60 per cent. of the dry weight
of the protoplasm consisted of proteins, about 20 per cent. of carbo-
hydrates and fatty substances, while the remainder consisted of
amino-acids and other organic acids, various organic bases and
inorganic salts. In different samples of protoplasm the relative
quantities of the substances of which it is composed vary; pro-
teins may form as little as 40 per cent. of the total dry matter. It
has been a matter of controversy whether cytoplasm is a single
definite substance peculiar to living matter, or whether it is com-
posed of a large number of relatively simpler substances. In the
elaboration of the former view the molecule or "biogen" is held
to consist of a stable central nucleus to which are attached side-
chains capable of undergoing various chemical transformations such
as oxidation and reduction, and to which the activities of the cell
are due (Verworn, 1903). The opinion that protoplasm comprises
a large number of different substances is more generally held to-
day. Thus Czapek (1911 a) remarks: "But we have to concede that
the chemical nature of protoplasm is not founded upon the pecu-
liarities of one particular substance which is characteristic of living
protoplasm. There are, we are certain of it, a great number of
constituents of protoplasm which form the substratum of cell-life."

Hopkins (1913) thinks the biogen molecule theory "as inhibitory to productive thought as it is lacking in basis." Bayliss (1915) regards the biogen theory as "an example of the efforts of a certain school of physiologists to explain by purely chemical laws, such as mass action, facts which admit of a simpler explanation, if physical phenomena are also taken into account."

We have to think of protoplasm not merely as an intimate mixture of a large number of substances, but as having a complex organisation so that the cell is rather an organ with an intricate minute structure, and at the same time different reactions can take place in different parts of the same cell. "Protoplasm is an extraordinarily complex heterogeneous system of numerous phases and components, continually changing their relations under the influence of electrolytes and other agents" (Bayliss, 1919). For a further discussion of this question reference may be made to the writings of Verworn, Czapek, Hopkins and Bayliss cited above.

Some parts of the protoplasm are clearly differentiated from the rest. The most important of these is the nucleus which has been observed in all plant and animal cells with very few exceptions. From its appearance in fresh cells and from its reaction to stains it obviously differs from the cytoplasm that encloses it. There is considerable evidence that it is much richer in nucleoproteins, which contain phosphorus, than the surrounding cytoplasm. The observations of Gaidukov and Price with dark-ground illumination point to the fact that the nucleus is in the gel condition. Kite (1915) and Chambers (1917) conclude on the contrary that the resting nucleus of the ovum is in the sol state. Price made out a definite limiting layer separating the nucleus from the cytoplasm, but he thinks it possible that this, the so-called nuclear membrane, may be no more than the surface of separation between the cytoplasmic hydrosol and the nuclear hydrogel. The intimate connection between cytoplasm and nucleus as regards cell activity is so well realised that it needs no further emphasis.

The plastids occur only in plants, and even then not in all plants. They are absent, for example, from the Fungi. Like the nucleus they are sharply differentiated from the rest of the cytoplasm and appear to contain much protein. In addition they often contain pigments (chlorophyll, xanthophyll and carotin) our knowledge of the composition of which is due to the persistent researches of Willstätter (see Willstätter and Stoll, 1913). According to

Price the chloroplasts of *Spirogyra, Elodea* and other plants are, like the nucleus, in the gel state.

The small granules occurring throughout the cytoplasm may be in some cases tiny non-living inclusions and in other cases small plastids or other living bodies. Chemically they may be composed of fat, glycogen, protein, etc., as shown by microchemical tests. Chambers divides them into microsomes and macrosomes, the former being very minute and considerably less than $1\,\mu$ in diameter, while the macrosomes vary from 2 to $4\,\mu$ in diameter and are circular, oval or polygonal in shape. The macrosomes are the alveolar spheres of Wilson (1899), with whom Chambers disagrees as regards the complete gradation from macrosomes to microsomes. Chambers regards the two sets of bodies as quite distinct; the macrosomes are the most easily injured, and the microsomes are the most resistant, of all cell structures. Into this controversy we need not enter here, nor yet into the question to what extent the microsomes are identical with mitochondria. Those interested should consult an article by Cavers (1914) and the very numerous papers on this subject published by Guilliermond (1911–1921) during recent years.

Whereas in animal cells the protoplasm frequently occupies the whole of the cell space, in plants this is the case only with meristematic cells. As the cell passes out of the meristematic condition small inclusions of less viscous consistency become obvious in the general mass of the cytoplasm. These are the vacuoles. As the cells grow older the vacuoles become larger and ultimately fuse into one large vacuole occupying the greater part of the cell cavity. Strands of cytoplasm traverse this vacuole and may even in the middle of the vacuole form a mass enclosing the nucleus. In the oldest cells which are still living even these strands may disappear and the cytoplasm is limited to a scarcely perceptible layer sur-. rounding the vacuole. In this case the nucleus lies in this lining layer of cytoplasm; it is never in direct contact with the vacuole.

The contents of the vacuoles are very varied. Occasionally solid particles may occur, as for instance, those of calcium sulphate in certain Desmids. Such particles have been observed by G. S. West in a number of green algae (Price, 1914). Apart from these are particles of ultra-microscopic dimensions which have been observed by Price. Undoubtedly, however, as the facts of osmotic pressure and turgor show, the contents of the vacuole consist chiefly of an aqueous solution of various substances. Thus in the bulb of the onion and in the root of the beet the substance is mainly sugar (De

Vries, 1884 *a*); in the bean, pea, buckwheat and maize it is said to be chiefly potassium nitrate (Copeland, 1897); in other plants potassium chloride may be present in considerable quantity, while in many cases organic acids are the principal substances present (De Vries, 1879, 1883; Kraus, 1886).

At the junction of the vacuole and the cytoplasm we have again a surface separating two immiscible phases with consequently properties differing from those of either phase, and it has become usual to regard that part of the cytoplasm directly surrounding the vacuole as a distinct layer spoken of as the internal plasmatic membrane, the tonoplast or the vacuole wall (De Vries, 1885). Price, by ultra-microscopic observation, has obtained indications of a definite layer in this location similar to that bounding the cytoplasm externally. To this question we shall have to return later.

Yet another complication is present in the case of plant cells, namely, the cell wall. Although this is absent in a few cases, nearly every plant cell is bounded by a firm envelope which thus separates the individual protoplasts from one another. The cell wall differs widely in morphological construction and chemical composition among different species and in different parts of the same plant, but with the exception of the Fungi one of the main constituents of the cell wall is cellulose, a carbohydrate, or more probably a group of carbohydrates, of high molecular weight. A number of other substances are present, these varying in different species and in different tissues of the same species. In the Fungi, including the Bacteria, the principal constituent of the cell wall is chitin. The cell wall of the young plant is always thin, but after the cell has reached its ultimate size, various morphological and chemical changes take place in the cell wall, for a description of which reference may be made to standard botanical text-books (for example, Sachs, 1875; Strasburger, 1903) and especially to the work of Mangin (1888–1893). It should be mentioned here that thickening of the cell wall may consist entirely of cellulose and allied substances, or the walls may be modified by the processes of lignification, suberisation or cutinisation, in which various substances grouped under the names lignin, suberin and cutin (see Priestley, 1921) are deposited in the cell wall and profoundly alter its properties. It should also be observed that when cell walls become thickened the thickening is rarely uniform. Scattered over the cell walls places occur at which thickening does not take place so that thin spots known as pits are dotted over the surface. The pits on the two sides of a wall separating two cells are

always opposite one another, so that at the pit there is only the original primary wall separating the two protoplasts. Chiefly through the researches of W. Gardiner (1884), A. Meyer (1896), Strasburger (1901) and A. W. Hill (1901) it is clear that fine cytoplasmic threads penetrate the pit membranes, and sometimes indeed the whole thickness of the cell wall, so that in spite of the presence of the cell wall there is actually a continuation of protoplasm throughout the plant. Cellulose walls readily absorb water, and in the living plant the cell wall is normally permeated with imbibed water. Cutinised and suberised walls are however more or less impermeable to water, and their principal function is to prevent loss of water from the surface of cells.

Such then is the system with which we are concerned in a consideration of the problems of permeability. This system is very varied, attaining its highest complexity in the adult plant cell. In the latter we have to recognise at least three phases, the cell wall, the protoplasm and the vacuole. Each of these moreover is itself a complex system, both the cell wall and protoplasm each containing a more watery phase and at least one other phase, while there is evidence that the cell sap in the vacuole may also contain a colloidal disperse phase as well as water with substances in pure solution. At the boundaries between outer medium and cell wall, cell wall and protoplasm, protoplasm and vacuole, there are separating layers which there is every reason to believe have different properties from the bulk of the phases they separate. Further, in both the cell wall and protoplasm, and perhaps also in the vacuole, we have at least two-phase and probably polyphase systems in which there are consequently relatively large surfaces of contact between the phases. The constituents of the different phases vary from plant to plant and from tissue to tissue in the same plant.

We thus see how much phase boundaries figure in the structure of the cell, and it is impossible to lay too much stress on the importance of surface phenomena in regard to cell permeability. Before passing on to problems of the cell itself it will therefore be necessary to discuss briefly the more important facts relating to surfaces, as well as other physical and physico-chemical principles with which acquaintance is necessary for a proper realisation of permeability phenomena.

CHAPTER III

SURFACE PHENOMENA

THE special properties of surfaces wherein these latter differ from the main mass of the phases they separate are related partly to mechanical and partly to electrical forces. Both of these certainly play a very important part in determining all permeability, and in consequence it is necessary to devote a little space here to a con-sideration of both mechanical and electrical phenomena connected with surfaces.
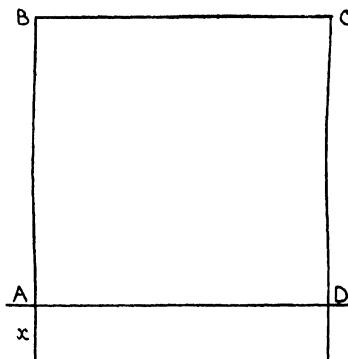

Fig. 1.

SURFACE PHENOMENA RELATED TO MECHANICAL FORCES.

The most obvious way in which the surface differs from the body of a liquid is in its behaviour as a thin stretched skin. This is most familiarly exemplified in the behaviour of films of soap solution. For instance if the rectangle enclosed by the wire frame $ABCD$ (fig. 1) is occupied by a thin film of soap solution terminated at its lower edge by the wire AD which can move freely along the vertical wires AB and DC, the film will tend to contract and it is

necessary to attach a definite weight to AD in order to keep it in position.

The surface is thus the seat of special forces. How these come to be present can be understood from the following considerations.

In the mass of the liquid we have an enormous number of molecules, each one of which is surrounded on all sides by numbers of other similar molecules uniformly distributed round it. The mutual attraction of the molecules therefore results in nothing more than keeping them pressed together, the pressure on any particular molecule being uniform in all directions. By the force of cohesion the liquid particles are kept together, and do not tend to separate as in the case of a gas. At the surface of the liquid the state of affairs is different. A molecule at the very surface is subjected to the attractive force of molecules in the interior of the liquid, but there is no similar balancing force at the exterior. This means that the surface layer is subjected to an inwardly directed pressure at right angles to the surface, a pressure which decreases rapidly in the direction away from the surface as the cohesive force of the molecules is more uniform in all directions.

This inwardly directed pressure at the surface has two results. In the first place as all liquids are compressible to a small extent the surface layer of liquid will be compressed; in the second place as all the molecules at the surface are pressed towards the interior the surface will tend to contract to its smallest possible area.

So far the surface has been spoken of as if there were nothing outside the liquid. As a matter of fact there must always be some substance in contact with the liquid. Now a force of attraction called adhesion exists between molecules of different substances, consequently the actual inwardly directed pressure at the surface is the result of the difference between the pressure due to cohesion of the liquid and that due to adhesion between the liquid and the external medium. Only when the cohesion and adhesion are equal the pressure will be zero.

We thus see that the surface is in a state of tension by which it tends to reduce its area to the least possible. The *surface tension* of a liquid is defined as the force acting on unit length in the plane of the surface. From what has already been said it is clear that the surface tension depends not only on the nature of the liquid itself, but also on the medium with which it is in contact. The surface tension for instance of the surface water/air will not have the same value as the surface tension of the surface water/alcohol. When the

surface tension of a liquid is spoken of it is generally the surface tension of the liquid against air that is meant.

The surface tension of a liquid, besides depending on the nature of the liquid and on the medium with which it is in contact, also depends on the temperature.

It is outside the scope of this work to describe the different methods used for the measurement of surface tension. For these reference may be made to standard text-books of physics (*e.g.* Poynting and Thomson, 1905; Winkelmann, 1908). The principles of the more ordinary methods consist respectively of: (1) the measurement of the height to which liquid will rise in a capillary tube; (2) the measurement of bubbles and drops (Quincke, 1870, 1871); (3) the measurement of the size of drops of the liquid as it issues from a narrow tube; (4) measuring the least pressure necessary to force bubbles of air from the orifice of a narrow tube dipping in the liquid (Jaeger, 1891 *a, b*); (5) the determination of the deformation produced in the cross-section of a stream of liquid issuing from an elliptical orifice; (6) forcing a stream of the liquid upwards through a small orifice and measuring the height to which it will rise; (7) the determination of the velocity with which waves travel over the surface of a liquid (Rayleigh, 1879); (8) observations of oscillations of a spherical drop of liquid (Lenard, 1887). These and other methods will be found described in physical text-books.

The following table shows the surface tension of a number of liquids in contact with air, as determined by the capillary method, at 20° C.

TABLE I

Surface tension of certain liquids in contact with air
at a temperature of 20° C.

Substance	Surface tension in dynes per cm.
Water	72·53
Acetic acid	23·46
Ethyl alcohol ...	22·03
Ether	16·49
Chloroform	25·88
Olive oil	35·4

In Table II are given the surface tensions of a number of liquids against different substances.

TABLE II

Values of the surface tension of the same liquid against different substances at 20° C. (Data from Quincke)

Liquid	Surface tension in dynes per cm. against		
	air	water	mercury
Water	80·97	0	41·77
Mercury ...	53·98	41·77	0
Alcohol ...	25·49	—	39·93
Chloroform ...	30·61	29·52	39·93
Olive oil ...	36·88	20·56	33·54

The values given in this table are due to Quincke (1870, 1871). His determinations are now generally regarded as somewhat high, and the figures given in Table I for surface tensions against air are lower in all cases than those found by Quincke. This does not affect the comparison of the surface tensions against different substances.

With increase in temperature the surface tension diminishes. Eötvos (1886) has propounded the following relation between surface tension and temperature

$$\frac{d\,(\sigma v^{\frac{2}{3}})}{d\theta} = -2\cdot1$$

where σ is the surface tension, v the molecular volume (*i.e.* molecular weight/density) and θ the temperature whatever the value of θ and whatever the substance. From this equation can be calculated the value at which the surface tension becomes zero; calculation shows this temperature differs very little from the critical temperature. (Cf. Poynting and Thomson, 1905.)

Since the surface of a liquid is in a state of tension, it follows that when it contracts energy is released, and conversely, when a surface is increased work has to be done against the tension. Consequently the surface is the seat of energy. Referring to the example of the soap film in fig. 1, since the surface tension is the force exerted by unit length of the surface, and since there are two surfaces to the film, the weight required to keep the wire AD in equilibrium must be $2\sigma.AD$.

Now if the film is stretched so that BA and CD are increased in length by a quantity x, the work done in stretching the film is

$$2\sigma.AD.x$$

or $2\sigma\omega$ where ω is the area by which the surface has been increased.

Consequently the potential energy of a surface is the product of the surface tension and the area. This quantity is called the *surface energy*.

It has been pointed out (Helm, 1887; Bayliss, 1915) that in general energy can be regarded as the product of two factors, an "intensity factor" of the nature of a force, and a "capacity factor" which is always a measure of size, such as mass, volume, area, length, etc. In the case of electrical energy for example, the intensity factor is difference of potential and the capacity factor strength of current. The capacity factors of two systems add together and the sum gives the capacity factor of the whole, the intensity factors cannot be added together. For instance in the case of heat, where the intensity factor is temperature, the sum of the temperatures does not give the temperature of the whole system. Differences in the intensity factor always tend to disappear. Thus two bodies in contact tend to come to the same temperature, or to the same potential. On the other hand there is as a rule no tendency for differences between capacity factors to disappear.

In the case of surface energy, the intensity factor is obviously the surface tension, and the capacity factor the area of the surface.

Now it is an important law of energetics that free energy always tends to reduce itself to a minimum, and hence the surface energy of a liquid will tend to diminish whenever this is possible. In most cases it is only the intensity factor which is capable of alteration to bring about reduction in the free energy, but in the case of surface energy both the intensity and capacity factors tend to diminish to the minimum possible value, that is the surface tension will reduce itself whenever possible and the surface will contract to the smallest possible. A reason for this has already been given in this chapter.

The tendency for surface tension to diminish is probably a principle of great importance in regard to permeability. In the case of two pure and immiscible liquids in contact there is no possibility for the surface tension to be reduced as this is a function of the nature of the liquids and the temperature. The case of a liquid that contains more than one component requires more consideration. In this case if the surface tensions of two components in the pure state are different, the surface tensions of mixtures of the two are intermediate between those of the pure liquids. Thus in the case of mixtures of ethyl alcohol and water, if the surface tension of pure water against air is taken as unity, that of ethyl alcohol against air is only 0·302. The curves in fig. 2 show how the addition of various alcohols to water lowers the surface tension of the latter. So far however it has not been possible to express the exact relationship

between surface tension and concentration of a solution algebraically.

In the case of a solution of ethyl alcohol in water the surface tension will obviously be reduced if the concentration of alcohol in the surface is raised. According to the principle first enunciated by Gibbs (1878, 1906) and emphasized by J. J. Thomson (1888), there will thus be a tendency for the alcohol molecules to migrate to the surface, so that the concentration in the surface layer is greater than
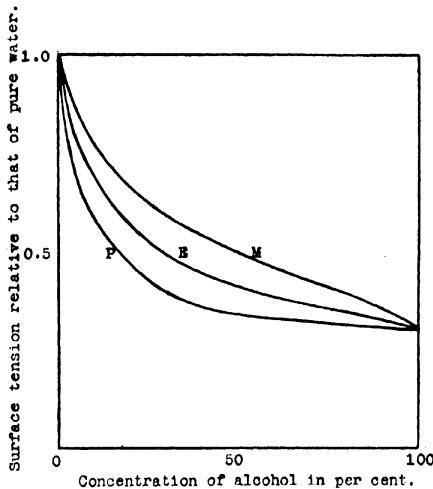

Fig. 2. Curves showing the relation between surface tension against air and composition of solutions of methyl (*M*), ethyl (*E*), and isopropyl (*P*) alcohols in water.

in the rest of the liquid. Opposing this tendency is that of the dissolved substance to diffuse from a place of higher to one of lower concentration. An equilibrium condition will thus be reached where these two opposed tendencies balance one another. It has been shown by Gibbs that when equilibrium is attained

$$\Gamma = -\frac{C}{RT} \cdot \frac{d\sigma}{dC}$$

where C is the concentration of the solute in the bulk of the solvent, Γ the excess of solute in the surface, $\frac{d\sigma}{dC}$ the rate of change of surface tension with concentration of the solute, T the absolute temperature, and R the gas constant.

S. P.

The accumulation of a dissolved substance at the surface forming the junction between two phases is called *adsorption*, and when it takes place purely as a result of the mechanical surface tension it is called *mechanical adsorption*.

It will be observed that accumulation of solute in the surface layer can only take place if the dissolved substance lowers the surface tension of the solvent. If on the other hand the dissolved substance should bring about an increase of the surface tension, the concentration of the surface layer will be less than that of the rest of the solution, and negative adsorption will result.

Attempts to verify the formula of Gibbs have been made by Lewis (1909) and by Donnan and Barker (1911) and an approximate agreement with the formula has been obtained in some cases. In the following table are shown the values found experimentally for Γ compared with the values obtained by determination of $\dfrac{d\sigma}{dC}$ and subsequent calculation from Gibbs's equation.

TABLE III

Values of Γ found and calculated.   (Lewis)

| | (In gm. per sq. cm.) | |
Substance	found	calculated
Sodium glycocholate	5×10^{-6}	7×10^{-8}
Congo red	$3 \cdot 7 \times 10^{-6}$	$1 \cdot 1 \times 10^{-7}$
Methyl orange	$5 \cdot 5 \times 10^{-6}$	$1 \cdot 2 \times 10^{-7}$
Sodium oleate	10^{-6}	10^{-8}
Sodium hydroxide	$1 \cdot 5 \times 10^{-7}$	$7 \cdot 5 \times 10^{-9}$
Caffeine	$3 \cdot 7 \times 10^{-8}$	$2 \cdot 4 \times 10^{-8}$
Sodium nitrate (kation)	$2 \cdot 5 \times 10^{-8}$	$4 \cdot 5 \times 10^{-9}$
Potassium chloride (kation)	5×10^{-8}	$1 \cdot 7 \times 10^{-9}$
,, ,, (anion)	10^{-9}	$1 \cdot 6 \times 10^{-9}$
Barium chloride (anion)	not $> 10^{-8}$	$1 \cdot 6 \times 10^{-9}$
Copper chloride (anion)	$3 \cdot 5 \times 10^{-8}$	2×10^{-9}
,, ,, (kation)	2×10^{-8}	2×10^{-9}

Lewis concludes that caffeine probably obeys Gibbs's Law quantitatively. In other cases the values found experimentally are always greater than those obtained by calculation with the exception of the anion of potassium chloride. Lewis thinks that some irreversible phenomenon such as gelatinisation at the surface may be the explanation of the discrepancy. As will be shown later in this chapter electrical phenomena are often present at a surface which add to the complexity of the matter.

Any substance dissolved in water lowers its surface tension against a solid or immiscible liquid, and this is usually the case also when

the solution is in contact with a gas. There are however some substances which increase the surface tension of water against air, among which âre most inorganic salts, although these lower it at thê interface between water and oil (Lewis, 1909).

An anomalous case of surface concentration is met with in the case of sugars. It appears that these substances in solution do not lower the surface tension of wâter. Nevertheless they are adsorbed at the surface to a slight extent (Herzog and Adler, 1908; Herzog, 1909; Rona and Michaelis, 1909). The explanation of this phenomenon offered by Rona and Michaelis is that the surface layer is in a state of compression (see p. 94) and the solubility of the dissolved substance is greater in this compressed layer than in the bulk of the solution.

In many cases the presence of the adsorbed material in the surface film of a liquid will increase the viscosity of the liquid at the interface. This may account for the formation of the rigid membranes observed by Ramsden. Similar membranes have also been obtained by Metcalf (1905) and Zangger (1908).

As we have seen in Chapter II we are concerned in the living cell with a decidedly viscous substance, and we can thus understand how the cytoplasmic "membrane" when damaged is immediately reformed, as has been often observed, *e.g.* by Chambers (1917) and Seifriz (1918).

Adsorption is in general a reversible process. In the case of such viscous fluids as solutions of proteins, soaps and bile acids, on the contrary, the process is irreversible.

Equations connecting the amount of adsorption at equilibrium with the concentration have been obtained empirically. Among the better known of these are those due to F. W. Küster (1894) and G. C. Schmidt (1894); the relation is most usually expressed in the form due to Freundlich (1907, 1909), which is

$$\frac{x}{m} = kC^{\frac{1}{p}}$$

where x is the mass of substance adsorbed by a mass m of the adsorbent, C is the concentration of the solution after adsorption has reached equilibrium, and k and p are constants. The value of p varies between 1·25 and 5, but it is very commonly about 2. This equation it will be observed may be written in the form

$$\log \left(\frac{x}{m}\right) = \frac{1}{p} \log C + K.$$

If then the logarithm of the amount of material adsorbed is plotted against the logarithm of the concentration at equilibrium, straight

lines will be obtained if the adsorption equation holds; this is shown to be approximately the case.

It follows from the adsorption equation that as the concentration of the solution increases the quantity adsorbed increases, but the quantity relative to the concentration decreases. For example, if finely divided charcoal is added to solutions of substances, adsorption generally takes place at the surface of the charcoal. Now if charcoal is added to a solution of acetic acid in water, when the concentration of the acetic acid at equilibrium is 0·018, the quantity adsorbed per unit mass of adsorbent is 0·467; when the equilibrium concentration is 2·79, the quantity adsorbed per unit mass of adsorbent is 3·76. That is, although $\frac{x}{m}$ in the adsorption equation has increased from 0·467 to 3·76, its value relative to the concentration of the solution, has decreased from 26 to 1·35.

As surface tension decreases with rise in temperature so adsorption also is less the higher the temperature. As the adsorption at a temperature $\theta + 10$ is thus a fraction of what it is at θ, adsorption has a *fractional temperature coefficient*, if the temperature coefficient of a process is regarded as the number by which the value of the process at one temperature has to be multiplied in order to give the value of the process at a temperature 10 centigrade degrees higher. The temperature coefficient is then generally denoted by the symbol Q_{10}. When the process, as in the case of adsorption, is lessened with rise of temperature, the temperature coefficient is less than unity. Such processes are often spoken of as having a *negative temperature coefficient*. This is not necessarily a misuse of the term negative, for by "temperature coefficient" is sometimes understood the quantity, or a multiple or fraction of a quantity, which has to be added to the value of a process for a rise of 1° C. This temperature coefficient is often denoted by the symbol α. It is unfortunate that the term "temperature coefficient" should be used in these two senses.

It must be noted that although adsorption decreases with rise of temperature, the rate at which adsorption is brought about increases. This is due to the fact that the rate of adsorption must depend on the rate at which the adsorbed substance can diffuse through the medium containing it. It is therefore to be expected that the rate of adsorption would have a temperature coefficient not far different from that for rate of diffusion, and this has been shown by Bayliss (1906) to be the case with adsorption of congo red by filter paper.

So far only pure substances, or solutions of one substance, have been dealt with. In the organism on the contrary we always have to deal with mixtures.

* In general if there are two substances which can be adsorbed they displace one another to some extent. As the concentration of one of the substances increases, more of it is adsorbed and displaces the other to a greater extent, but relative to the concentration the amount of displacement decreases with increasing concentration. An exception to this is found in those viscous substances which tend to form rigid membranes at the surface. The presence of a viscous solute has no influence on the adsorption of a solute of low viscosity.

Another peculiarity of such substances is that the extent of adsorption depends not on their concentration, but on the absolute amount present. It is not clear how far the peculiar properties of such substances are due to high viscosity, low diffusivity or colloidal nature. Sugar as is well known considerably increases the viscosity of water, but the adsorption of sugar follows the ordinary laws and is reversible.

So far we have considered the consequences of the tendency of surface tension to reduce itself to a minimum. A few words are now necessary regarding the tendency of the area of the surface to diminish as far as possible. This is illustrated by the fact that liquids free from external forces always take up a spherical form, while if a liquid is divided into droplets scattered through another liquid of the same density, and with which the first liquid is immiscible, the droplets tend to unite into larger ones and finally into one single sphere.

In the case of living matter of a gel nature, however, we have a substance which possesses a certain amount of rigidity. Any change in form of droplets of such a substance will therefore be opposed by the resistance offered by the rigidity of the substance to change of shape, and a condition of equilibrium will be reached when these two tendencies are equal.

The tendency of surface tension in gels is therefore to bring about the coalescence of separated droplets. This union is called agglutination when microscopic particles coalesce into particles visible with the naked eye, and coagulation when the particles that unite are ultra-microscopic or very finely microscopic. Agglutination and coagulation may therefore be produced either by an increase in surface tension or by a decrease in rigidity of the dispersion medium.

ELECTRICAL PHENOMENA AT SURFACES.

So far we have considered the surface effects which are attributable to purely mechanical phenomena. The conditions at surfaces are however actually often more complex than has so far been indicated owing to the very general presence of electrical forces at the interface between two phases.

We know for instance that if a metal is immersed in a solution of one of its salts a difference of potential between the metal and solution results, and that the existence of this potential can be explained by the tendency of metallic ions to pass into solution. When this takes place the metallic ions give to the solution a positive charge, leaving the metal plate correspondingly negatively charged. The solution of the metal can therefore only proceed until the mutual attraction due to the difference in potential is balanced by the tendency of the metal to go into solution; *i.e.* by the electrolytic solution pressure of Ostwald which is proportional to the ratio between the concentrations of atoms in the metal and ions in the solution. The difference of potential is given by the expression

$$RT \log \frac{P}{p}$$

where P is the electrolytic solution pressure, p the osmotic pressure of the ions in solution, T the absolute temperature and R the gas constant.

The same considerations hold for hydrogen as for a metal and are the basis of the well-known electrometric method now so universally employed in physiology for measurement of hydrogen ion concentration.

In the case of the surface between a solid electrolyte and its solution similar considerations hold, though the matter is complicated by the presence of two ions. In this case the potential at the interface between the solid and liquid phases is given by

$$RT \log \frac{P^{\cdot}}{p^{\cdot}} \left[= RT \log \frac{p'}{P'} \right]$$

where $P^{\cdot}$, P' are the electrolytic solution pressures of the kation and anion respectively and $p^{\cdot}$, p' are the osmotic pressures of kation and anion in the liquid phase.

In general, for the potential difference due to one ion at the interface between any two immiscible phases, we have for the value of the potential the expression

$$\frac{RT}{nF} \log \frac{c_1}{c_2} + K$$

where c_1 and c_2 are the concentrations of the ion in the two respective phases, n the valency of the ion, F the electric charge of a monovalent gram-ion, and k a constant. It is outside the scope of this work

to describe how these formulae are derived; those interested should consult the original work of Nernst (1889, 1892) and Haber (1908) and the general account of electrical phenomena at surfaces given by•Michaelis (1914). For the application of these formulae to biological phenomena reference may be made to the papers of Beutner (1912 *b*, 1913 *c*, *d*) and Loeb and Beutner (1912).

It should be emphasized that the difference of potential (phase potential) arises from the unequal partition coefficients of the two ions between two phases, and is not connected with the different mobilities of the two ions. A difference of potential due to this latter cause (diffusion potential) arises when two solutions containing the same ions but in different concentrations come into contact. Diffusion takes place and if the mobilities of the two ions are different one ion will diffuse faster than the other and a difference of potential will thus result.

It appears however that the sign of the charge should be the same in whichever of the two ways the difference of potential arises, for in the latter case the solution takes the charge of the more mobile ion and in the former case it takes the charge of the more soluble one, and it appears that the more soluble ions are also the more mobile (Michaelis, 1914). Probably the electric charge at most surfaces can be accounted for in one of the preceding ways, but there are cases, as for instance that at the surface of aniline in contact with water (Ellis, 1912) where the aniline is negatively charged, although one would expect it to be positively charged as it feebly dissociates into the slightly mobile aniline ion $C_6H_5.NH_3$ and the very mobile hydroxyl ion. Although explanations have been offered of such cases (Lewis, 1910) the problem cannot be regarded as solved.

We may now pass on to a consideration of how adsorption is affected by electrical phenomena.

In the first instance we may consider a case of an electrolyte partly dissociated into its constituent ions. There will then be in solution the kation, the anion and the undissociated molecule all with their characteristic constants in regard to the adsorption equation, so that they tend to be adsorbed to different extents. Any difference in adsorption of the kation and anion must however be very slight as this would result in a potential difference between the surface and the interior of the liquid. An equilibrium position would soon be reached where the force of attraction between the excess of oppositely charged ions would prevent any further separation. Such differences in potential due to inequalities of differently adsorbed ions are called *adsorption potentials* (see Freundlich, 1909).

The charge at a surface may itself be responsible for adsorption. By the principle that free energy will always reduce itself to a minimum, the electrical energy at a surface will tend to diminish whenever this is possible. Now if a surface is the seat of a negative charge, the deposition of any particle or ion carrying a positive charge will reduce the electrical energy of the surface, and such deposition will therefore tend to take place. That this is actually the case has been shown by Perrin (1904–1905), Bayliss (1906), Lachs and Michaelis (1911) and others. For instance Bayliss has shown that a negatively charged surface such as that of filter paper will adsorb large quantities of an electropositive substance such as night blue, but only a trace of substance carrying a negative charge such as congo red. In some cases of such *electrical adsorption* the charge on the surface may actually be reversed; it seems likely that in these circumstances we are dealing with a complex effect in which both mechanical and electrical adsorption are involved.

Adsorption may obviously have an effect in regard to chemical reactions taking place at the surface. Thus if two substances which react together are adsorbed on the surface of a third, combination will take place on the surface of this third substance, which itself may remain unaltered. In a similar way reactions may take place if two substances mutually adsorb one another as in the case of barium hydroxide added to colloidal silica (van Bemmelen, 1910). A white substance is precipitated containing both substances, and from the mixture barium silicate slowly forms.

In such cases the active mass of reacting substances is the number of molecules adsorbed to the surface, and this number is proportional to the extent of the surface. Hence in such cases the rate of reaction is proportional to the surface. This is for example very generally the case in enzyme actions (see Bayliss, 1914).

Finally it must never be forgotten that all the phenomena of surfaces occur not only at the obvious surfaces of the cell, but throughout the colloidal substance of which the protoplasm is composed. Protoplasm is a heterogeneous system of more than one phase and throughout the system whenever there is a boundary surface between the disperse phase and the dispersion medium the surface phenomena described in this chapter must occur. A general acquaintance is assumed here with the properties of colloids. Reference may be made to the works of Hatschek (1913), Taylor (1915), Bayliss (1915, 1918), Zsigmondy (1917, 1920) and Wolfgang Ostwald (1909, 1917, 1920) in which colloids are treated from various standpoints.

CHAPTER IV

DIFFUSION

IF two gases are separated by a partition which is subsequently
removed, the molecules of one gas pass into the space occupied
by the other until a condition of equilibrium is reached in which the
two gases are equally distributed throughout the whole space. A
similar diffusion takes place in the case of two miscible liquids, except
that owing to the internal friction or viscosity the rate of equili-
bration is much slower. Similarly, if the two liquids brought into
contact consist of solutions of different substances in the same solvent,
then diffusion proceeds until the solutes are equally distributed
throughout the whole of the liquid; or if the solvent contains the
same dissolved substance but in different concentrations in the two
liquids, diffusion proceeds until the solute is equally distributed
through the whole of the solvent, always supposing that no external
force is operative.

Phenomena involving the diffusion of gases do indeed occur in
the plant, as in the processes of carbon-assimilation, respiration
and transpiration. But directly any gas reaches the surface of a cell
any further passage of the molecules of the gas through the cell
takes place in an aqueous medium, and in problems of permea-
bility it is with diffusion through a liquid that we are concerned,
and in most cases with diffusion through an aqueous medium.

The first systematic researches on diffusion of liquids were made
by Thomas Graham (1850), who investigated the diffusion of a
variety of substances dissolved in water. He showed that the quantity

of salt diffusing through unit area of the solvent depended on the concentration of the salt, on the nature of the salt, and on the temperature. The greater the difference of concentration, the more salt diffused in unit time, and the higher the temperature the faster the rate of diffusion.

The subject was later investigated by Fick (1855) who, by comparison of the problem with that of the conduction of heat worked out by Fourier, propounded the well-known equation which has come to be known as Fick's law. This equation is

$$dC = -D\frac{\partial c}{\partial x}dt,$$

where C is the quantity of salt passing through unit area in a time dt at a point x where the concentration gradient (*i.e.* the rate of change of concentration with distance) is $\frac{\partial c}{\partial x}$. The value of D is constant for any particular substance in any definite concentration and at a definite temperature and is called the coefficient of diffusion for the substance, or the diffusivity. It is thus the quantity of salt diffusing across unit area in unit time when the concentration gradient is unity (that is, when two cross-sections of the liquid at unit distance apart differ by unity in their concentrations).

Fick's law states therefore that the quantity of salt diffusing across unit area is proportional to the coefficient of diffusion, to the concentration gradient and to the time of action. This law has been abundantly verified since the time of Fick. For the solution of the differential equation reference may be made to the work of Fourier (1822, 1878) or to the various more modern works dealing with Fourier's equations (for example, Carslaw, 1906; Weber and Riemann, 1910–1912). Reference may also be made to a paper by Kelvin (1889, 1890) in which are given the curves showing the relation between concentration of solution at a point distance x from the initial surface of contact between water and a saturated solution, the position of the point x, and the time of action.

It will be sufficient here to consider only the simplest case, that of a salt diffusing into a cylindrical column of water. If we regard the column of water as infinitely long, the solution of Fourier's equation for this case is

$$u = u_0\left\{1 - \frac{2}{\sqrt{\pi}}\int_0^q e^{-q^2}dq\right\},$$

where

$$q = \frac{x}{2\sqrt{Dt}},$$

and u is the concentration at a point in the cylinder distant x from the initial surface of contact between water and the solution, and after a time t has elapsed from the commencement of diffusion, u_0 is the concentration of the original solution at the mouth of the cylinder and D is the coefficient of diffusion. The value of

$$\frac{2}{\sqrt{\pi}} \cdot \int_0^q e^{-q^2} dq$$

for different values of q is given in tables of the probability integral (see, for example, Peirce, 1910).

It follows from this equation that if u is constant, that is, if we consider the march forward into the column of water of a particular concentration of diffusing substance, that q must also be constant. Hence

$$\frac{x}{2\sqrt{Dt}} = \text{constant},$$

or, stated in words, the distance any particular concentration of salt has reached is proportional to the square root of the time of action and to the square root of the coefficient of diffusion. This relation has been repeatedly verified (Coleman, 1887, 1888; Chabry, 1888; Voigtländer, 1889).

Various methods have been devised for the measurement of coefficients of diffusion. These may be classified as follows:

(1) Those depending on the estimation after a certain time by chemical analysis of the concentration of the solution occupying different layers in the vessel in which diffusion is proceeding.

(2) Methods in which the concentration of different layers of solution is determined from estimations of the density of the solution.

(3) Methods based on the estimation of the rate at which substances go into solution.

(4) Indicator methods in which the presence of an indicator in the liquid shows when a certain concentration of the diffusing substance is reached at any particular place.

(5) Electrical, and (6) optical methods.

For a description of these various methods reference may be made to the article on Diffusion by Waitz (1908) in Winkelmann's *Handbuch der Physik*. An electrical conductivity method is described by Haskell (1908).

In Table IV are given the coefficients of diffusion of a number of substances in water. These values are taken from the results of Thovert (1901, 1902), Scheffer (1888), Schuhmeister (1879) and Heimbrodt (1903, 1904), and the calculations made by Stefan (1879) from the data of T. Graham.

TABLE IV

Coefficients of Diffusion of Various Substances

Substance	Concentration in gm.-mols. per litre	Temperature in °C.	Coefficient of Diffusion in $\dfrac{cm.^2}{sec.} \times 10^{-5}$	Observer
HCl	3·2	19·2	4·50	Thovert
,,	0·02	19·2	2·45	,,
HNO$_3$	3·9	19·5	2·85	,,
,,	0·02	19·5	2·45	,,
H$_2$SO$_4$	2·85	18·0	1·85	,,
,,	0·005	18·0	1·51	,,
KOH	3·9	13·5	2·81	,,
,,	0·02	13·5	1·95	,,
NaOH	3·9	12·0	1·14	,,
,,	0·02	12·0	1·30	,,
KNO$_3$	3·9	17·6	1·03	,,
,,	0·02	17·6	1·48	,,
KCl	2·95	17·5	1·85	,,
,,	0·02	17·5	1·57	,,
NaCl	3·9	15·0	1·18	,,
,,	0·02	15·0	1·09	,,
CaCl$_2$	1·22	9·0	0·83	Scheffer
,,	0·0468	9·0	0·79	,,
K$_2$SO$_4$	1·5	10	0·87	Schuhmeister
,,	0·95	19·6	0·92	Thovert
ZnSO$_4$	2·95	19·5	0·38	,,
,,	0·025	19·5	0·58	,,
MgSO$_4$	3·23	10	0·31	Scheffer
Ethyl alcohol	3·75	11	0·52	Thovert
,, ,,	0·05	11	0·85	,,
Glycerol	1·75	10·14	0·35	Heimbrodt
,,	0·125	10·14	0·41	,,
Citric acid	0·0303	4·5	0·39	Scheffer
Sucrose	1·97	18·5	0·15	Thovert
,,	0·97	18·5	0·28	,,
Albumin	—	13	0·073	Graham-Stefan

From the numbers given in this table it will be observed that the coefficient of diffusion varies considerably for different substances. It is also dependent upon the concentration of the diffusing substance, upon the temperature and upon the nature of the medium through which diffusion is taking place. In the following discussion, except where expressly stated otherwise, the medium is always assumed to be water.

As regards the influence of the nature of the substance on the coefficient of diffusion, generally speaking the diffusivity is high for electrolytes and substances of low molecular weight, while it decreases with increasing complexity of the molecule, being lower for substances like sugars and very low for colloidal substances. Of the commoner electrolytes, acids and alkalies diffuse most rapidly, and

in general electrolytes with the highest diffusivity have the highest electrical conductivity.

Mathematical formulae have been evolved to express these relations in the case of diffusion of non-electrolytes. Thus Sutherland (1905) and Einstein (1905, 1906) derived the formula

$$D = \frac{RT}{N} \cdot \frac{1}{6\pi\eta\rho},$$

where D is the coefficient of diffusion, R is the gas constant, T the absolute temperature, N the Avogadro constant (that is, the number of molecules in one gram molecule), η the viscosity of the solvent, and ρ the radius of the diffusing molecules which are assumed large in comparison with those of the solvent. Sutherland showed, however, that should the molecules of the solvent be large in comparison with those of the solute, the relation more nearly approximates to

$$D = \frac{RT}{N} \cdot \frac{1}{4\pi\eta\rho}.$$

Von Smolukowski (1906) obtained a similar expression but with a different constant, his formula being

$$D = \frac{64}{27} \cdot \frac{RT}{N} \cdot \frac{1}{6\pi\eta\rho}.$$

A general confirmation of this formula experimentally has been made by Svedberg and Andreen-Svedberg (1909, 1911) who could not, however, decide whether the constant in the equation of Einstein, Sutherland and von Smolukowski is 1 or $64/27$.

The relation between molecular size and coefficient of diffusion has been stated in another form by Exner (1867, 1874, 1877) for the case of gases. Exner's conclusion is expressed by the equation

$$D \sqrt{M} = k,$$

where D is the coefficient of diffusion, M the molecular weight and k a constant. This relation has been extended to the case of non-electrolytes by Öholm (1910), who confirmed it experimentally for a number of sugars and other substances, and who used it to determine the molecular weight of dextrin from the coefficient of diffusion of the latter. A third formula has been proposed by Herzog (1910), namely

$$D\eta \sqrt[3]{Mv} = \text{constant},$$

where v is the specific volume and the other symbols have the signification already assigned to them. This relation is stated to hold for a number of non-electrolytes (Padoa and Corsini, 1915).

There seems thus good reason for concluding that the coefficient of diffusion is related in inverse fashion to the complexity and magnitude of the molecule.

With regard to the connection between coefficient of diffusion and electrical conductivity, Nernst (1888) put forward the following equation to express the relation between diffusivity and ionic mobilities:

$$D = RT \cdot \frac{uv}{u + v},$$

where u and v are the ionic mobilities of anion and kation, and the other symbols have the same signification as before. Nernst himself realised that this formula could only be approximately correct as the diffusivity varies with concentration, nor does the formula take into account the diffusion of the undissociated part of the molecule.

To allow for the influence of concentration on diffusion, Wiederburg (1899) modified Nernst's formula as follows:

$$D_c = RT \left(\frac{2uv}{u + v} - w \right) \left(1 + \frac{4C}{K} \right)^{-\frac{1}{2}} + w,$$

where D is the coefficient of diffusion at a concentration c, K the dissociation concentration at the concentration and temperature in question, and w the molecular mobility.

Arrhenius (1892) obtained good agreement between experiment and the equation

$$D_c = D_\infty \left(1 + \frac{2BC}{RT} \right) \left(1 - \frac{\alpha C}{2} \right)^2,$$

where D_∞ is the coefficient of diffusion at infinite dilution, α a constant depending on the viscosity, and B a constant.

Arrhenius found the coefficient of diffusion of hydrochloric acid and of potassium hydroxide increased with increasing concentration, but that the reverse was the case with acetic acid, sodium hydroxide and sucrose. Scheffer also found an increase of diffusivity with increasing concentration in the case of hydrochloric acid; with sodium and potassium chlorides he found no notable change, while with sodium nitrate, sodium thiosulphate and silver nitrate the diffusivity diminishes with increasing concentration. A careful investigation of the influence of concentration on diffusivity of a number of electrolytes has been made by Öholm (1905); the data in Table V are taken from among his results. Data with regard to the influence of concentration on diffusion of glycerol, urea, sodium chloride and hydrochloric acid are given by Heimbrodt (1903, 1904).

TABLE V

Influence of Concentration on the Diffusivity at 18° C.
(Data from Öholm)

Concentration in gram-equivalents per litre	Coefficient of Diffusion in $\dfrac{cm.^2}{sec.} \times 10^{-5}$			
	NaCl	KCl	HCl	KOH
0·01	1·35	1·69	2·69	2·20
0·02	1·33	1·66	2·64	2·19
0·05	1·32	1·63	2·61	2·17
0·10	1·29	1·61	2·58	2·15
0·20	1·27	1·58	2·55	2·13
1·00	1·24	1·54	2·57	2·15
2·00	—	1·53	—	2·19
2·8	1·23	—	—	—
3·6	—	1·55	—	—
5·5	1·23	—	—	—

That the rate of diffusion of substances increases with rise of temperature was observed by Graham. Since these early observations quantitative determinations on the effect of temperature on the diffusivity have been made for a number of substances. Weber (1879) found that for every rise in temperature of 1° C. the coefficient of diffusion of zinc sulphate in water increased by about 0·026 of its value at 18° C. Later Seitz (1898) confirmed this value and obtained a similar one (0·024) for zinc acetate. A number of substances were examined by de Heen (1884) at temperatures varying from 15° to 60° C. In Table VI are shown the values he obtained for the coefficient of diffusion at any temperature θ, this being measured in centigrade degrees distant from 60° C. taken as zero, and reckoned as positive *downwards* from 60° C.

TABLE VI

Influence of Temperature on the Coefficient of Diffusion
(Data from de Heen)

Substance	Coefficient of Diffusion in $\dfrac{cm.^2}{sec.} \times 10^{-5}$
$MgSO_4$	0·85 (1 − 0·0119 θ)
KNO_3	3·07 (1 − 0·0127 θ)
NaCl	2·72 (1 − 0·0121 θ)
Na_2HPO_4	2·06 (1 − 0·0128 θ)
K_2CO_3	1·63 (1 − 0·0127 θ)

It will be noticed that the relative increase in diffusion for the same rise in temperature is almost independent of the substance

among those examined, although the absolute coefficient of diffusion varies greatly. These results agree well with those obtained for zinc salts to which reference has already been made.

On theoretical grounds Nernst (1888) propounded the formula

$$D_\theta = D_{18} \left[1 + \alpha \left(\theta - 18\right)\right]$$

to express the influence of temperature on the coefficient of diffusion, D_θ and D_{18} being the coefficients of diffusion at $\theta°$ C. and $18°$ C. respectively, and α a constant having the value 0·026 for neutral salts and 0·024 for acids.

Assuming the correctness of Nernst's general formula, the value of the temperature coefficient α in Nernst's equation has been calculated by Öholm from measurements of the coefficient of diffusion of a number of electrolytes (Öholm, 1902, 1905) and non-electrolytes (Öholm, 1910) at temperatures between $0°$ and $20°$ C. The values found by him are summarised in Table VII.

TABLE VII

Temperature Coefficient of Diffusivity of a number of substances
(Data from Öholm)

Substance	Temperature Coefficient
HCl	0·019
NaCl	0·025
KCl	0·0235
LiCl	0·027
KI	0·0235
NaOH	0·023
KOH	0·021
Acetic acid	0·028
Sucrose	0·032
Lactose	0·032
Maltose	0·032
Raffinose	0·032
Arabinose	0·044
Dextrin	0·035
Nicotine	0·016

Although there are obvious exceptions, Öholm concludes that in general the temperature coefficient of diffusivity is less the greater the diffusivity. Thus hydrochloric acid, with its very high coefficient of diffusion, has a very low temperature coefficient of diffusivity, while the slow diffusing sugars have temperature coefficients considerably higher than those of neutral inorganic salts.

It should be noted that it is assumed by all workers that the relation between diffusivity and temperature is a linear one. While this may be approximately true over the small temperature intervals with which Öholm worked, it is scarcely likely to be an exact

representation of the facts over a wide temperature interval; it may be sufficiently accurate over the temperature range with which we are generally concerned in living organisms.

We have so far considered the laws of diffusion in a medium which at equilibrium is homogeneous. In the organism, as we have seen, we have to deal very largely with heterogeneous systems. As the simplest case of a heterogeneous system we may consider two immiscible liquids separated by a phase boundary. That such systems actually occur in the living cell there can be little doubt. If a solute is soluble in both the liquids, its distribution when diffusion has proceeded to equilibrium will not be uniform throughout the system; on the contrary, the solute generally distributes itself unequally between the two solvents. This phenomenon was investigated by Berthelot and Jungfleisch (1869–1872) and later by Nernst (1891). It is found that if a substance has the same molecular complexity in the two solvents the ratio of the concentration of the solute in one solvent to its concentration in the other is a constant whatever the concentration. Thus if c_1 is the concentration in one solvent and c_2 the concentration in the other solvent,

$$\frac{c_1}{c_2} = K,$$

where K is a constant and called the *partition coefficient* or the *distribution ratio*. If several solutes are present together each one distributes itself between the solvents according to its own partition coefficient independently of the others; that is, K is independent of the presence of other solutes.

If the solute should undergo polymerisation in one of the solvents, the partition law becomes modified to

$$\frac{c_1}{c_2^{1/n}} = K,$$

where n represents the number of molecules associated together in one of the solvents.

With the law of partition coefficients may be compared the law governing the distribution of a substance between a solvent and an adsorbent at equilibrium. The quantity $\frac{x}{m}$ in the adsorption equation is of the nature of a concentration, and if for this we write c_1, and if for the concentration of the solute at equilibrium we write c_2, the adsorption equation becomes

$$\frac{c_1}{c_2^{1/p}} = K,$$

which represents the distribution of the solute between solvent and adsorbent. The similarity of this equation to that of the partition law is obvious.

The principle involved in the partition law has been applied with great success to the separation of substances which otherwise have so far been found inseparable, or are only separable with great difficulty. The separation of the four pigments of the green leaf by Willstätter and Stoll (1913) is almost entirely based on the different partition coefficients of the four pigments and the substances with them, in the commoner organic solvents.

It is now necessary to consider how far the laws governing the rate of diffusion are modified in more complex systems.

The case of an electrolyte diffusing in a solution of another substance instead of in pure water has been investigated by Öholm (1912). He found that the presence of a non-electrolyte such as glycerol or sugar appreciably reduces the rate of diffusion. In the following table are given the values obtained by Öholm for the coefficients of diffusion of potassium chloride in solutions of non-electrolytes of different concentrations.

TABLE VIII

Diffusion of Potassium Chloride in Solutions of Sucrose and Glycerol of Various Concentrations
(Data from Öholm)

Substance	Concentration in gram-mols. per litre	Coefficient of Diffusion at 18° C. in $\dfrac{\text{cms.}^2}{\text{sec.}} \times 10^{-5}$
Sucrose	0·0	1·535
,,	1·5	0·486
,,	2·0	0·255
Glycerol	5·0	0·501
,,	7·48	0·201

We now come to the important question of the diffusion of substances in colloidal systems. The diffusion of various substances in gels has received a considerable amount of attention, partly because of its obvious bearing on physiological problems (cf. Chapter II), and also because it lends itself more readily in some ways to investigation than diffusion in free liquids.

It was concluded by Graham (1861) that sodium chloride diffused as rapidly through gelatine as through water. De Vries (1884 c) on the other hand concluded from observations of the colour that

potassium chromate diffuses more slowly through 4 per cent. gelatine than through water, and Coleman (1888), examining the diffusion of a number of electrolytes in gels, also concluded that the presence of the jelly brings about a retardation in the rate of diffusion. Hagenbach (1898) came to a similar conclusion in regard to the diffusion of gases in gelatine. Henri and Calugareanu (1903), from observations on the diffusion of a number of dyes in water and 1, 3 and 5 per cent. gelatine, concluded that the rate of diffusion was the same through the gelatine as through water if the former had not set to a gel, but that if this had taken place, the rate of diffusion was slower. Bechhold and Ziegler (1906) concluded that electrolytes and non-electrolytes diffuse through gelatine and agar gels more slowly than through water.

Öholm (1913) compared the diffusion of potassium chloride from a normal solution into gels of 2, 5 and 10 per cent. gelatine. His results, summarised in the accompanying table, indicate very clearly the retarding effect on diffusion of the gelatine.

TABLE IX

Diffusion of Potassium Chloride from a Normal Solution into
Gels of Gelatine of Various Concentrations
(Data from Öholm)

Concentration of Gelatine in per cent.	Kind of Gelatine	Coefficient of Diffusion at 20° C. in $\frac{cm.^2}{sec.} \times 10^{-5}$
2	Kahlbaum (golddruck)	1·52
5	,, ,,	1·44
10	,, ,,	1·31
2	A brand from Dundee	1·50
5	,, ,, ,,	1·41
10	,, ,, ,,	1·28

If these numbers are compared with those given in the preceding table it will be observed that, as Öholm points out, a gelatinous body affects the diffusion of an electrolyte in just the same way as any other non-electrolyte. The observations of Stiles and Adair (1921) indicate that the presence of gelatine decreases the rate of diffusion of sodium chloride progressively with increase in concentration of the gelatine. There can be no doubt that the coefficient of diffusion of electrolytes is less in gels of gelatine than in water.

Voigtländer (1889) made a study of diffusion in agar-agar gels varying in concentration from 1 to 5 per cent. and ound that the

rate of diffusion was practically independent of the concentration or the gel. Sodium chloride, magnesium chloride and hydrogen chloride were among the substances examined, but the researches of Stiles and Adair have failed to confirm this conclusion in the case of sodium chloride, which appears to diffuse more slowly the greater the concentration of agar-agar, so that agar-agar behaves in the same way as gelatine in reducing the rate of diffusion.

The consequence of Fick's law in the case of linear diffusion in one direction, namely that $x = C \sqrt{Dt}$ where C is a constant (see p. 139), has been confirmed for diffusion into gels by Coleman (1888), Chabry (1888), Voigtländer (1889), von Fürth and Bubanović (1918), Stiles (1920) and Adair (1920), these various authors using a great variety of diffusing substances and gels of gelatine, gelose and agar-agar. The law holds equally well when the gel contains an indicator to mark the progress of a layer of definite concentration provided the concentration of the indicator does not approach too near to that of the diffusing substance (Stiles, 1920).

There are few observations on the influence of temperature on the coefficient of diffusion in gels. The results of Voigtländer on diffusion in agar-agar gels indicate a greater increase in diffusivity brought about by an increase in temperature from 20° C. to 40° C. than that brought about by an increase from 0° C. to 20° C. Voigtländer employs the equation

$$D_\theta = D_0 \, (1 + \beta\theta)^2$$

to express the relation between the coefficient of diffusion and temperature, D_θ and D_0 being the coefficients of diffusion at θ° C. and 0° C., and β a constant. It will be observed that as the values of β are small (0·01 to 0·02) in comparison with unity, when θ is also small then approximately

$$D_\theta = D_0 \, (1 + 2\beta\theta),$$

since the term $\beta^2\theta^2$ is small in comparison with $1 + 2\beta\theta$. Consequently, when θ is small the relation between the temperature and the coefficient of diffusion is approximately a linear one as assumed by de Heen, and by Öholm following Nernst. But when θ is large the term $\beta^2\theta^2$ is not negligible, and the coefficient of diffusion increases more rapidly with rise in temperature the higher the temperature. The results of Stiles and Adair on the diffusion of sodium chloride in agar-agar gels at temperatures from 0° C. to 40° C. support this latter assumption rather than the opinion of a linear temperature coefficient.

The observations of Chabry (1888) show that the course of diffusion of an electrolyte through a dead animal tissue is similar to its diffusion through a gel. Prismatic pieces of hyaline cartilage stained with orcein were fitted into a tube and any space between the tissue and the tube were filled with a transparent oil. The penetration of acid into the tissue was then followed by the indicator method in the same way as in the case of a gel, and it was found that the consequence of Fick's law, that $\frac{x}{\sqrt{t}}$ = constant, held in this case as well as in the cases of diffusion in water and in gels. The value of the constant was however found to be considerably less than with diffusion into a gel.

The diffusion of crystalloids through colloidal systems such as gels and dead organic tissue apparently proceeds in the same way as through water, although less rapidly. But the diffusion of colloids differs strikingly in a simple liquid and in a colloidal system. It is well known that Graham drew the fundamental distinction between colloids and crystalloids on account of the non-diffusibility of the former through membranes of parchment and other colloidal materials, while crystalloids diffused readily through such membranes. The diffusibility or non-diffusibility of a substance or group of substances through membranes is however generally not an absolute property of the membrane; it is a matter of degree. Thus colloidal membranes which are permeable to crystalloids do not let them all through equally readily. This is a matter of obviously great importance in relation to permeability of living tissue and requires to be considered in some detail; it therefore forms the subject of the next chapter.

CHAPTER V

THE PERMEABILITY OF MEMBRANES

THAT colloids are generally incapable of penetrating into a colloidal system was mentioned at the end of the last chapter, and the incapacity of colloids to diffuse through colloidal membranes[1] is the phenomenon which led Graham to distinguish between colloids, substances which are incapable of passing through such membranes or which do so very slowly, and crystalloids, which pass through these membranes readily. By utilising this principle Graham was able to separate colloids from crystalloids, and so introduced the method of separation and purification of colloids known as dialysis.

The permeability of a membrane depends on the composition of the membrane, but the latter is not equally permeable to all substances. For example, caouchouc allows pyridine to pass through it while water is kept back. When a membrane is in contact with a solution the permeability may, and generally does, differ as regards the solvent and the solute. Thus parchment paper allows water to pass readily, but the solute in the case of an aqueous solution of sucrose diffuses through the membrane very slowly. In speaking of the permeability of a membrane it is thus necessary to define the system with which the membrane is in contact.

A membrane which allows a substance to pass through it readily is said to be *permeable* to the substance, while one which does not allow the substance to pass through it is said to be *impermeable* to the substance.

Although colloids generally cannot diffuse through membranes of colloidal substances, it must be realised that this distinction between colloids and crystalloids is by no means absolute. By

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[1] The term "membrane" may be limited to thin solid structures, while the term "film" is used for thin layers of liquid. In biology it is often difficult or impossible to distinguish between solid membranes and liquid films, and the term "membrane" will therefore be used to include films.

varying the membrane and the substance, practically any degree
of permeability may be found. Particularly interesting in this
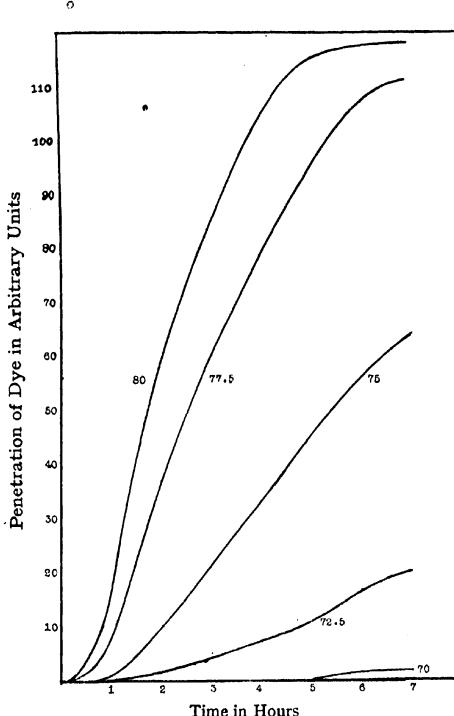
Fig. 3. Curves to illustrate the penetration of methylene blue through collodion
thimbles of graded permeability. (Constructed from the data of W. Brown.)

respect are the observations of W. Brown (1915) who describes
a method by which membranes of collodion may be prepared
of any degree of permeability within certain wide limits. Brown's
method consists in preparing completely air-dried membranes
of collodion which are highly impermeable. The membranes are
then immersed in solutions of alcohol in water for a suitable
time. After washing in water the membranes then exhibit a per-
meability which increases with increase in the concentration of
alcohol employed.

As an example of the degree of variation in permeability which
may be obtained by this method an experiment made by Brown

may be cited. Membranes in thimble form of different degrees of permeability were prepared as described by treatment with 70, 72·5, 75, 77·5 and 80 per cent. alcohol respectively. The thimbles were filled with a solution of methylene blue, and immersed in vessels containing distilled water. The amount of diffusion was estimated at daily intervals by colorimetric estimations of methylene blue in the external solution. The curves shown in Fig. 3 are constructed from the data given by Brown. They show what a wide range in permeability is obtained by grading collodion membranes in aqueous solutions of alcohol ranging in concentration from 70 to 80 per cent. As practicable membranes can be obtained by the use of alcohol solutions ranging in concentration from 0 to 97 per cent. it will at once be clear what an extremely wide range of permeability can be obtained at will. The following table gives the values found by Brown for what he calls the "alcohol index" of a number of substances. The alcohol index of a substance is defined as the number which represents the strength of alcohol required to produce a membrane which just prevents diffusion of the substance.

TABLE X

Alcohol Indices of a Number of Substances. (Data from W. Brown)

Substance	Alcohol Index
Water	0
Sodium chloride	0
Potassium permanganate	30–40
Picric acid	35–40
Copper sulphate	60–70
Potassium oxalate	60–70
Sodium sulphate	60–70
Bismarck Brown	65–
Methylene Blue	70–
Neutral Red	72·5–75
Safranin	75–77·5
Dextrin	85–87·5
Starch	90
Aniline Blue	92
Litmus (neutral)	93
Congo Red	96
Night Blue	>96

In a later paper Brown (1917) extends his method for obtaining membranes of graded permeability to other material (gelatine, agar-agar) and a number of grading liquids. Other methods of obtaining membranes of graded permeability are described by Bechhold (1907), Bigelow and Gemberling (1907), Schoep (1911) and Walpole (1915).

When a solution is in question a membrane may be equally permeable to both solvent and solute, or it may be impermeable to both. More frequently the permeability to the two components

differs, and generally, in the case of solutions of crystalloids in water, the membranes are permeable to both water and the solute, and more readily to water.

The different rates of diffusion of solvent and solute through a membrane were shown in the first half of the nineteenth century by Dutrochet (1827, 1828, 1832 a, b) and Vierordt (1848) who examined the diffusion of aqueous solutions of salt through membranes of pig's bladder. The water passes through the membrane faster than the dissolved salt, this difference in rate depending on the nature of the salt, its concentration, and, as shown by Graham (1854), on the nature of the membrane.

Graham's discovery of the impermeability of certain membranes to colloids led Moritz Traube to search for a membrane which, while permeable to water, should be impermeable not only to colloids but to crystalloids. Such a membrane, permeable to the solvent but impermeable to any solute, may be termed a *semi-permeable* membrane. Although a perfect semi-permeable membrane has never been manufactured, some of the precipitation membranes prepared by Traube (1867) are rather near approximations to one. The best known of these, and the one which has probably been most used, is that of copper ferrocyanide, which is obtained in the form of a gel when solutions of potassium ferrocyanide and copper sulphate come into contact. For most purposes these membranes are too delicate if unsupported, and so they are usually precipitated in the wall of a porous pot, a device due to Pfeffer (1877). Other precipitation membranes are those of glue-tannic acid, copper tannate, lead tannate, ferric ferrocyanide, copper silicate and tin silicate. These membranes, like the collodion membranes already mentioned, are graded in their permeability.

Tammann (1892) investigated the penetration of a number of salts through precipitation membranes of copper ferrocyanide and zinc ferrocyanide. He found the permeability of the two membranes was the same as regards the simple salts he investigated though not in regard to dyes. Traube (1867) had found the copper ferrocyanide membrane permeable to potassium, sodium and ammonium chlorides, and impermeable to barium chloride and nitrate, calcium chloride, potassium sulphate and ammonium sulphate. Tammann, on the contrary, found the membrane permeable to all these salts except calcium chloride. His findings in regard to permeability of the copper ferrocyanide membrane to a number of salts of the alkali and alkaline earth metals are collected in the following tables.

TABLE XI

Permeability of Precipitation Membranes of Copper Ferrocyanide to a Number of Sulphates. (Data from Tammann)

Salt	Quantity passing through membrane
$(NH_4)_2SO_4$	small quantity
K_2SO_4 $\}$ Na_2SO_4 $\}$	smaller quantity
Li_2SO_4	a trace
$MgSO_4$	none

TABLE XII

Permeability of Precipitation Membranes of Copper Ferrocyanide to Chlorides, Bromides and Nitrates of Metals of Alkalies and Alkaline Earths. (An asterisk indicates that traces only pass through the membrane.) (Data from Tammann)

Kation	Anion		
	Chloride	Bromide	Nitrate
Ammonium $\}$ Potassium $\}$ Sodium $\}$ Lithium $\}$	All penetrate the membrane in large quantities; much more so than the corresponding sulphates.		
Barium	perm.	perm.*	perm.*
Strontium	perm.*	perm.*	imperm.
Calcium	imperm.	imperm.	imperm.
Magnesium	imperm.	imperm.	imperm.

From quantitative investigations on the diffusion of acids through the copper ferrocyanide membrane, Tammann came to the conclusion that the rate of passage of the acid through the membrane was chiefly dependent on the degree of dissociation of the acid, the more dissociated the acid the more rapid its passage through the membrane.

An investigation by Walden (1892), the results of which were published almost contemporaneously with those of Tammann just quoted, agrees on the whole with these latter. Walden found, however, that membranes of copper ferrocyanide and zinc ferrocyanide had distinctly different permeabilities. Altogether the permeabilities of eleven precipitation membranes to a large number of acids and salts were examined. The membranes employed are recorded in the following table. All these membranes were impermeable to tannin, while all were permeable to the following anions when bound to alkali metal kations (K, Na, ?Li, NH_3): F, Cl, Br, I, CN, CNS, ClO_3, ClO_4, BrO_3, IO_3, NO_2, NO_3, formate, acetate, trichloracetate, isobutyrate, valerianate, salicylate, sulphate, thiosulphate, AsO_3, B_4O_7 (the two silicate membranes were not examined in the case of the last four anions). The results with other ions are shown in Table XIII.

TABLE XIII

Permeability of Various Precipitation Membranes to Anions bound to Alkali Metals (K, Na, ?Li, NH$_4$) (p permeable, i impermeable). (Data from Walden)

Membrane	PO$_4$	AsO$_4$	SO$_4$ (SeO$_4$)	CrO$_4$	P$_2$O$_7$	Oxalate Ferrocyanide Ferricyanide Cobaltocyanide Silicate
Glue-tannic acid	p	p	p	p	p	p
Ni$_3$(CoCy$_6$)$_2$	p	p	p	p	p	i
Ni$_2$FeCy$_6$	p	p	p	p	p	i
Co$_3$(CoCy$_6$)$_2$	p	p	p	p	i	i
Co$_2$FeCy$_6$	p	p	p (i)	i	i	i
Cd$_3$(CoCy$_6$)$_2$	p	i	i (p)	i	i	i
Nickel silicate	.	.	i	i	i	i
Cobalt silicate	.	.	i	i	i	i
Cu$_3$(CoCy$_6$)$_2$	i	i	i	i	i	i
Zn$_2$FeCy$_6$	i	i	i	i	i	i
Cu$_2$FeCy$_6$	i	i	i	i	i	i

In Table XIV are given Walden's results as regards the permeability of precipitation membranes to a number of positive ions bound (generally) to halogens.

The significant fact to be noted from Walden's results is that these membranes form a perfectly regular series in regard to their permeability to the substances examined, glue-tannic acid being the most permeable, and allowing the passage of everything investigated except alkali tannates and halides of (?) cadium, zinc and manganese, while copper ferrocyanide is the least permeable, the other membranes being intermediate in their permeability.

From these results and those obtained with a large number of acids, Walden concluded that the penetrability of substances depends not so much on the number and weight of the atoms composing their molecules, as on the nature and arrangement of the constituent atoms. Thus sulphate and thiosulphate have the same number of atoms in the molecule, but precipitation membranes are much more permeable to the latter than to the former. The same is the case with sodium acetate and sodium oxalate. In general, while both electrolytes and non-electrolytes may be able to pass through a membrane, acids and salts containing at least one univalent ion diffuse through most easily. Potassium and ammonium chlorides, bromides and iodides pass through membranes with nearly equal ease. With other bases the diffusion of halides is greater the greater the atomic weight of the base.

TABLE XIV

Permeability of Various Precipitation Membranes to Positive Ions
bound (in most cases) to Halogens. (Data from Walden)

Membrane	Permeable to	Impermeable to
Glue-tannic acid	Alkalies, Cu, Ba, Mg, Fe", Ni, Co, Hg", Pb, Al	?Cd, Zn, Mn"
Ni_2FeCy_6	Alkalies, Mg, Ba, As	Ni, Co, Cu, Zn, Cd, Mn
$Ni_3(CoCy_6)_2$	Alkalies, Ba, As, Sb	Ni, Co, Cu, Zn, Cd, Mn
$Co_3(CoCy_6)_2$	Alkalies, Ba, Sb	Fe, Ni, Co, Cu, Zn, Cd
$Cd_3(CoCy_6)_2$	Alkalies, Ba, Sb	Cd, Co, Ni, Zn, Cu, Mn
Co_2FeCy_6	Alkalies	Mg, Ca, Sr, Ba (slightly permeable), As, Co, Ni, Cu, Zn, Cd, Mn
$Cu_3(CoCy_6)_2$	Alkalies	Fe, As, Ca, Ba, Zn, Cu, Co, Ni, Mn, Cd
Zn_2FeCy_6	Li, Na, K, Tl, NH_4, $C_2H_5NH_3$, $(C_2H_5)_2NH_2$ (slightly permeable)	Ca, Mg, As, Zn, Cu, Mn, Co, Ni, Cd, $(C_2H_5)_3NH$
Cu_2FeCy_6	Li, Na, K, Tl, NH_4	Be, Mg, Cu, Sr, Ba, As

The effect on permeability of the addition of neutral salts to
acids varies according to the acid. In some cases such addition is
without effect (for example with hydrochloric and trichloracetic
acids), in other cases there results a small hindrance to diffusion (as
with sulphuric acid), while in other cases the addition of salts has a
very significant effect (as with tartaric, glyceric and acrylic acids).

Tammann's observations on the penetration of 17 different dyes,
including salts of colour bases, sodium salts of sulphonic acids, and
acids, through three precipitation membranes, namely, glue-tannic
acid, zinc ferrocyanide and copper ferrocyanide, showed seven
exceptions to the regularity of the general order of permeability.
Thus of the 17 dyes examined, eleven passed through the glue-tannic
acid membrane, seven through the zinc ferrocyanide membrane and
five through the copper ferrocyanide membrane. Nevertheless,
fuchsin chloride, for instance, was able to penetrate the glue-tannic
acid membrane and the copper ferrocyanide membrane, but not the
zinc ferrocyanide membrane, although in general the zinc ferrocya-
nide membrane is more permeable than the membrane of copper
ferrocyanide. An inverse case is that of cotton blue, to which the
membrane of zinc ferrocyanide is permeable, but which can penetrate
neither the copper ferrocyanide membrane nor the glue-tannic acid
membrane, which is, in general, the most permeable of the three
membranes examined.

It is to be noted in regard to the case of the permeability of the

copper ferrocyanide membrane to fuchsin chloride, that according to Meerburg (1893) the dye is only able to penetrate the membrane until the latter is completely impregnated with dye.

Membranes then differ among themselves in regard to their permeability, and the permeability of any particular membrane is different to different substances. Generally the order of permeability to different substances is the same in the case of different membranes but there are exceptions to this rule. It would appear that membranes are much more permeable to halides than to sulphates and also considerably more permeable to salts of monovalent metals than to those of divalent metals. In some cases the permeability depends on the degree of dissociation of the substance but this is not by any means a universal rule.

Wilhelm Ostwald (1890) pointed out that in the case of a dissociated salt the membrane need only be impermeable to one ion of a salt in order to prevent both ions from passing, for on account of the electrostatic attraction of the oppositely charged ions the permeable ion will only travel to such a distance that its tendency to diffuse balances the electrostatic attraction. If, however, the membrane should separate the salt solution not from pure solvent, but from another salt both ions of which can penetrate the membrane, the case is more complicated. (See Chapter VIII.)

It will be observed that very little quantitative work has been done on the permeability of membranes, and consequently exact data as to the influence of temperature and other factors on permeability are for the most part wanting. The influence of pressure on the passage of water through membranes has however been the subject of investigation by several workers, *e.g.* Schmidt (1856), Pfeffer (1877), Sebor (1904), Bigelow (1907) and Bartell (1911), from whose observations, and especially from those of Bigelow and Bartell, it appears that the rate of passage of water through membranes is proportional to the pressure. The membranes investigated by these different authors include various animal membranes (Schmidt), membranes of collodion and parchment paper (Bigelow), porcelain (Bigelow, Bartell), and copper ferrocyanide (Pfeffer, Sebor, Bartell).

The influence of temperature on the permeability of membranes of collodion to water at constant pressure was investigated by Bigelow and his results extended to porcelain by Bartell. The conclusion of these investigators is that the permeability expressed as the quantity of water passing through unit area of membrane in unit time, is given by the relation $M_\theta = M_0 (1 + 0.03368\theta + 0.000221\theta^2)$,

where M_θ, M_0 are the permeabilities of the membrane at temperatures, $\theta°$, $0°$ respectively. As this formula is that connecting the viscosity of liquids at different temperatures the authors conclude that the change in permeability of a membrane at different temperatures is nothing more nor less than a measure of the change of viscosity of the liquid.

There remain to be considered the various theories put forward to explain the differential permeability of membranes. There are two well-defined theories which are usually described as the sieve theory and the solution theory.

The sieve theory was first put forward by Traube (1867) who attempted to explain the semi-permeability of his precipitation membranes by supposing them to act as "atom-sieves" (more properly "molecule-sieves"), so that molecules below a certain size pass through, while those above this limiting size are kept back.

The experiments of Tammann and Walden already cited, as well as those of Kahlenberg (1906), do not wholly favour the sieve theory. Nevertheless, the theory has found in recent years a considerable number of supporters, *e.g.* Sutherland (1897), Perrin (1900). In an investigation on the passage of dyes through parchment paper Biltz (1910) found that the rate of passage of dye through the membrane depended very definitely on the dimensions of the molecule of dye. Thus when the number of atoms in the molecule did not exceed 45 the dye passed readily through the membrane; as the number of atoms in the molecule increases from 45 to 70 the rate of penetration through the membrane is slower and slower, and when the number of atoms in the dye molecule is greater than 70, the membrane is impermeable to the dye.

Also Bayliss (1915) points out that the difficulties in the way of the theory presented by Tammann's experiments in which the order of penetration of different substances varies with the membrane, can to a large extent be explained away.

Contrasted with the sieve theory is the solution theory of permeability, which may be traced back to the writings of Liebig (1849) and L'Hermite (1855). According to this theory substances pass through the membrane if they are soluble in the membrane, and are held back if they are insoluble in it. L'Hermite illustrated the theory with the "three liquid layers" experiments, one of which may be quoted as an example. Layers of water, castor-oil and alcohol were superposed in a cylinder, the castor-oil thus forming the layer separating water and alcohol. The alcohol is soluble in the castor-oil and passed through the middle layer

composed of it (equivalent to a membrane) into the water. Similar experiments were performed with other systems of three liquids. The application of these experiments to the permeability of membranes is obvious, and the solution theory of permeability has found many supporters, *e.g.* Nernst (1890), Kahlenberg (1906), and Overton (1897), to whose work more detailed reference will be made later.

From his work on the influence of pressure and temperature on permeability, Bigelow (1907) concludes that the passage of water through membranes of collodion obeys Poiseuille's formula for the movement of water through capillary tubes, and consequently that the passage of substances through such membranes is capillary in nature. The inconsistent results first obtained with porcelain were shown by Bartell (1911) to be due to mechanical clogging of the pores, a phenomenon which M. Traube thought he had been able to bring about in the case of precipitation membranes and which he adduced as evidence in support of the sieve theory. By clogging the pores of a membrane, the latter may become impermeable to substances to which it was formerly permeable. It has been pointed out by Bayliss (1915) that such clogging can also take place as a result of adsorption. The views of Bigelow seem at first sight to approach the sieve theory, but Bigelow shows that the capillary theory reconciles the opposed sieve and solution theories. In the case of porcelain the passage of water undoubtedly takes place through capillary pores, while in the case of water diffusing through collodion there is good evidence that the water passes through intermolecular spaces, *i.e.* dissolves in the membrane. But the phenomenon is essentially the same in the case of these and other membranes. Consequently Bigelow concludes that the rate of passage of liquids through molecular interstices is expressible by the same laws which formulate the rate of passage of liquids through capillary tubes.

This view, that capillary phenomena only differ in degree but not in kind, from chemical phenomena, can be traced back to L'Hermite.

Apart from the two definite theories of permeability here discussed, there has been suggested a third theory, which may be spoken of as the chemical theory, according to which the membrane is supposed to combine chemically with the substance to which it is permeable. This reaction is supposed to be reversible so that on the far side of the membrane the compound of membrane and diffusing substance breaks down with the result that the diffusing substance is set free on the far side of the membrane. This theory has been applied chiefly to cell problems, but has not found the support accorded to the sieve and solution theories.

CHAPTER VI

OSMOTIC PRESSURE

THE diffusion of a liquid through a membrane is termed osmosis. As long ago as 1748 it was observed by Nollet that when a bladder filled with alcohol was immersed in water the bladder swelled, while when a bladder full of water was immersed in alcohol the bladder shrank owing in each case to the water passing through the membrane more rapidly than alchohol.

Similarly, if an aqueous solution of, say, sucrose is contained in a bladder impermeable to the solute but permeable to the solvent, on immersing the bladder in water, the latter alone passes through the membrane into the bladder; which is in consequence distended, and may indeed ultimately burst if the solution inside is sufficiently concentrated. Thus when a pure solvent and solution are separated by a semi-permeable membrane, the pure solvent tends to pass through the membrane so as to dilute the solution, for this is the only way in which diffusion can take place so as to bring about equalisation of concentration throughout the system. Consequently when solution and pure solvent are separated by a semi-permeable membrane a hydrostatic pressure is produced which forces water through the membrane from solvent to solution.

The osmotic pressure of a solution is defined as "the equivalent of the hydrostatic pressure produced when the solution and solvent are separated by a perfectly semi-permeable membrane; or as the equivalent of the excess pressure which must be imposed on a solution in order to prevent the passage into it of solvent through a perfectly semi-permeable membrane" (Findlay, 1913).

DETERMINATION OF OSMOTIC PRESSURE

Osmotic pressure is easily demonstrated by the familiar laboratory experiment in which a solution of sucrose is placed in an inverted thistle funnel, the mouth of which is closed by a parchment membrane and which is then placed in a vessel of pure water. The membrane being impermeable to sucrose, water passes through the membrane and consequently the level of liquid rises in the tube of the thistle funnel. This process continues until the pressure of the height of liquid in the funnel is equal to the osmotic pressure forcing water into the funnel. The principle involved in this experiment furnishes the basis for the direct method of measuring osmotic pressure. For a detailed description of this method and of the indirect methods of measuring the osmotic pressure of a solution, reference may be made to the monograph by Findlay already cited. Here it will be sufficient to record the various methods which are employed. These are as follows:

1. *Direct Method.* In the direct method of measuring osmotic pressure a semi-permeable precipitation membrane of copper ferrocyanide is deposited in the wall of a porous pot into the mouth of which is sealed a manometer which serves for the determination of the pressure produced. Great difficulties are involved in this method, and the original apparatus devised by Pfeffer (1877) has since been greatly improved by later workers, especially by Morse and his co-workers (1901–1912) and by Berkeley and Hartley (1904, 1906 *a*).

The indirect methods of determining osmotic pressure depend on the relations which exist between osmotic pressure and various physical properties. According to the particular property measured the indirect methods may be called the vapour pressure method, the freezing point method, the boiling point method, and the critical solution temperature method.

2. *Vapour Pressure Method.* The vapour pressure of a liquid is lowered by the presence of a dissolved substance. A number of equations have been obtained by different investigators for the relation between vapour pressure and osmotic pressure. The formula of Spens (1906) is probably an exact enough approximation for most purposes. This equation is

$$Pv_s = sp \log_e \frac{p}{p'},$$

where P is the osmotic pressure, p and p' are the vapour pressures

of pure solvent and solution respectively, s the specific volume of the vapour and v_s is the increase in volume of a large mass of solution on addition of unit mass of solvent. For other formulæ, more or less exact, Findlay's book may be consulted, or the original papers of Arrhenius (1889), Berkeley and Hartley (1906 b), Porter (1907, 1908), Callendar (1908), and Berkeley, Hartley and Burton (1908). The actual method of measurement of the vapour pressure depends on bubbling an indifferent gas through the solution, the amount of vapour taken up by the gas being proportional to the vapour pressure. In the original experiments of Walker (1888) the gas is allowed to bubble first through the solution and then through the pure solvent. The amount of vapour taken up in the second operation is proportional to the difference between the vapour pressures of pure solvent and solution.

3. *Freezing Point Method.* As is well known the presence of a dissolved substance lowers the freezing point of a solution. A number of formulæ have been derived also in this case to express the relation between lowering of freezing point and osmotic pressure, for a discussion of which the reader is again referred to Findlay.

4. *Boiling Point Method.* Just as determinations of the lowering of the freezing point of a solution due to a dissolved substance can be used to calculate the osmotic pressure of a solution, so the elevation of the boiling point resulting from the presence of a solute can be used to the same end. The method has, however, been little employed for this purpose.

5. *Critical solution temperature method.* Certain liquids are miscible in all proportions only above a certain temperature. Below this temperature they are miscible only to a limited extent, and consequently if a liquid is cooled below this temperature one of the liquids separates out as a distinct phase. This critical solution temperature is altered by dissolved substances in proportion to the osmotic pressure of the solute. A method of measuring osmotic pressure based on the work of Timmermans (1907) in this relation has been employed for physiological purposes by Atkins and Wallace (1913) to whose work further reference may be made.

Methods for *comparing* osmotic pressures by the use of living cells and tissues were devised by de Vries (1884 a, b). These are the plasmolytic methods and the tissue tension method. They will be more conveniently described in a later chapter dealing with the water relations of the plant cell.

RELATION OF OSMOTIC PRESSURE TO TEMPERATURE
AND CONCENTRATION

The osmotic pressure of an aqueous solution depends on the dissolved substance, its concentration and the temperature. In the case of dilute solutions, Pfeffer (1877) concluded that the osmotic pressure
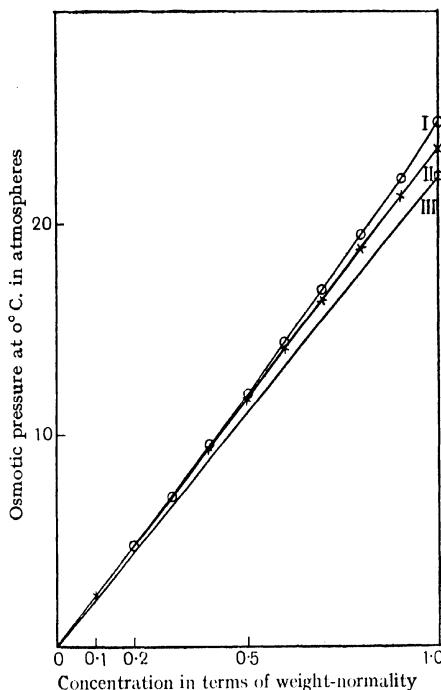
Fig. 4. Relation between concentration of a solution and its osmotic pressure. I. Measured values for sucrose. II. Measured values for glucose. III. Theoretical values calculated from the equation $PV = nRT$. (Data from Morse and collaborators.)

is proportional to the absolute temperature and to the concentration, that is,

$$P = K \cdot CT,$$

where P is the osmotic pressure, C the concentration and T the absolute temperature.

Now the concentration is the reciprocal of the volume occupied by unit mass of the solute, so that the relation becomes similar in

form to that connecting pressure, volume and temperature of a gas, namely $$Pv = KT.$$

Van't Hoff (1887) further showed that the equation is not merely the same but identical with the gas law equation and that if v is measured as the volume of one gram-molecule, then K has the same value as the gas constant R in the equation

$$Pv = RT,$$

which in consequence holds also for dilute solutions.
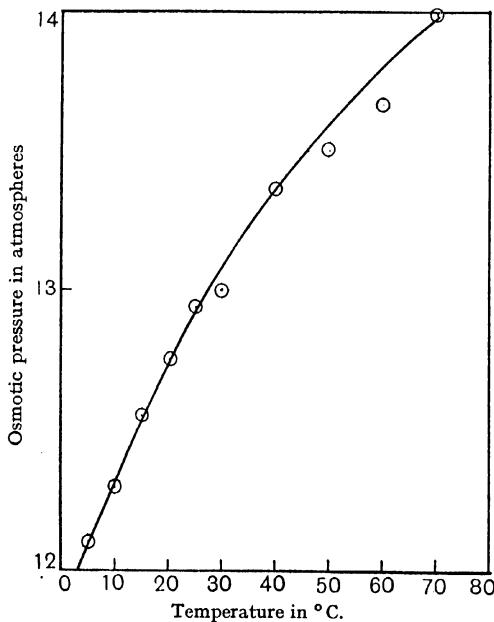
Fig. 5. Relation between temperature and osmotic pressure of a 0·5 weight normal solution of sucrose.

If V is the volume of the solution and n the number of gram molecules of solute present in the volume V, the equation becomes in consequence $$PV = nRT.$$

How near an approximation this is in dilute solutions will appear from Figs. 4 and 5. In Fig. 4 is shown in graphical form the relation between concentration of solutions of sucose and osmotic pressure at constant temperature, and in Fig. 5 is shown the relation between

temperature and osmotic pressure at constant concentration in the case of sucrose. These curves are constructed from the figures provided by Morse and his co-workers. For a large mass of further data reference may be made to the original papers of these workers.

It will be observed that in concentrations up to normal the proportionality between concentration and osmotic pressure holds quite well, but it is to be noticed that the measured osmotic pressure is always higher than that calculated from the gas law. This difference
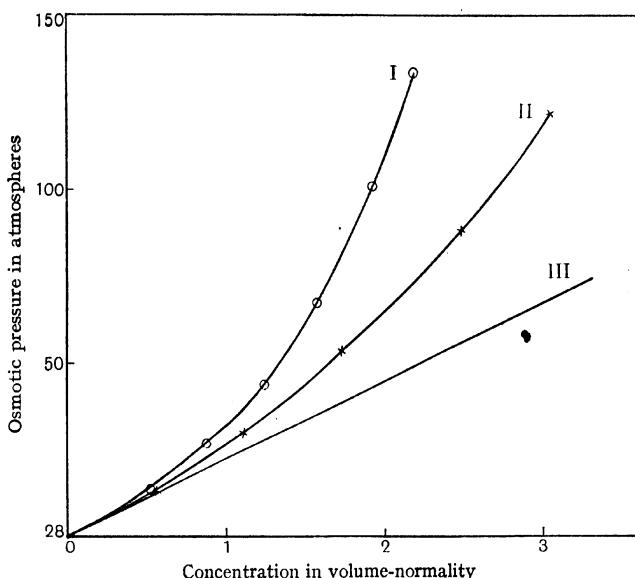
Fig. 6. Relation between concentration and osmotic pressure of solutions of sucrose and glucose. I. Sucrose at 0° C. II. Glucose at 0° C. III. Theoretical curve at 0° C. (Data from Berkeley and Hartley.)

between observed and calculated values is greatest at the higher concentrations, and in very high concentrations this divergence increases, as the curves in Fig. 6, constructed from the data obtained by Berkeley and Hartley, clearly show. The relation between temperature and osmotic pressure is also not one of strict proportionality over large temperature intervals. It has been pointed out by Morse that the divergence between observed and calculated values is considerably lessened even at high concentrations if it is assumed that the osmotic pressure is equal to the pressure the solute would exert

as a gas occupying the volume of the solvent only and not the volume of the solution. Even so, the divergence is not eliminated, and consequently various formulæ have been put forward to relate more exactly the concentration, temperature and osmotic pressure of solutions. A consideration of these is outside the scope of this work, and the reader is referred to the authorities already cited. It may, however, be emphasized that the simple van't Hoff law is valid for dilute solutions.

It has been pointed out that the deviations of concentrated solutions of sucrose from the behaviour of "ideal" solutions may to a large extent be explained on the assumption that sucrose is hydrated in solution, that is, that with each molecule of sugar a certain number of molecules of water become associated. In concentrated solutions this may materially reduce the quantity of solvent and so increase the concentration of solute, and consequently the osmotic pressure, in the way actually observed. In dilute solutions, where the quantity of solvent is very large in proportion to the quantity of solute the influence on concentration of solute is negligible (Nernst, 1911; Findlay, 1913).

MEASURED VALUES OF THE OSMOTIC PRESSURE OF SUCROSE.

A consideration of the work of Berkeley and Morse and their respective collaborators is sufficient to indicate the very great care and persistence required for the exact determination of osmotic pressures. Fortunately the substance most carefully studied, namely, sucrose, is one of particular importance for the student of permeability in plants, as will appear in a later chapter. It will therefore be useful to record here a few of the values obtained by Morse and regarded by him as established with a reasonable degree of certainty. In the following table are therefore given the values for the osmotic pressure of sucrose in different concentrations at temperatures most generally encountered in working with plants. For values at other temperatures, as well as for values of the osmotic pressure of solutions of glucose and mannite reference must be made to the summary of his work made by Morse (1914).

TABLE XV

Osmotic Pressures of Aqueous Solutions of Sucrose.
(Data from Morse)

Concentration in weight-normalities	Osmotic pressure at various temperatures			
	0° C.*	10° C.	20° C.	30° C.
0·1	2·462	2·498	2·590	2·474
0·2	4·723	4·893	5·064	5·044
0·3	7·085	7·335	7·605	7·647
0·4	9·443	9·790	10·137	10·295
0·5	11·895	12·297	12·748	12·978
0·6	14·381	14·855	15·388	15·713
0·7	16·886	17·503	18·128	18·499
0·8	19·476	20·161	20·905	21·375
0·9	22·118	22·884	23·717	24·226
1·0	24·826	25·693	26·638	27·223

OSMOTIC PRESSURE OF ELECTROLYTES

So far this discussion has been confined to sugars and the remarks already made may be regarded as applying generally to crystalloidal non-electrolytes. There are, however, two other groups of substances of the greatest importance which require some special treatment; these are electrolytes and colloids.

In the case of electrolytes the osmotic pressure is found to be considerably higher than the theoretical pressure calculated from the gas law. This is correlated with the dissociation of the molecule into its constituent ions. If each particle, either ion or molecule, is regarded as exercising the same osmotic pressure then agreement with the general law is found to exist in the case of dilute solutions of electrolytes as well as dilute solutions of non-electrolytes.

The formula

$$PV = nRT$$

must therefore be modified in the case of electrolytes to

$$PV = n\,(\alpha\beta + 1 - \alpha)\,R\,.\,T,$$

where α is the degree of dissociation of the electrolyte and β the number of ions into which the molecule dissociates.

Electrolytes have not so far received the attention that has been devoted to sucrose with regard to exact measurement of the osmotic pressure. The investigation of electrolytes is fortunately being undertaken by Morse and some results have already been obtained with lithium chloride. The values obtained by de Vries for a number of electrolytes and non-electrolytes will be referred to in a later chapter.

OSMOTIC PRESSURE OF COLLOIDS

Whether colloids exert an osmotic pressure or whether they do not is a question which has been the subject of some discussion. Now as colloidal properties are due either to the large size of the molecule, or to the aggregation of a number of molecules, we should expect colloids to exhibit the same behaviour in regard to osmotic pressure as other substances. The influence of association of molecules in the case of hydration has already been touched upon.

It was Starling (1896, 1899) who first showed experimentally that a colloid exerted an osmotic pressure. Blood serum was filtered through gelatine under pressure, and in this way the crystalloids were separated from the colloids, which alone were held back. The filtrate was then separated from unfiltered serum by a gelatine membrane, and the hydrostatic pressure developed was measured; any osmotic pressure produced is then attributable to the colloids. In this way it was shown that the colloids of blood serum exerted an osmotic pressure of 3 to 4 cm. of mercury. Further determinations of the osmotic pressure of colloids have since been made by other observers, notably by Hüfner and Gansser (1907) who found the osmotic pressure of hæmoglobin corresponds to the molecular weight calculated from chemical data (cf. also Reid, 1905), and it may now be assumed with confidence that colloids do actually exert a definite osmotic pressure. Owing to the large size of the molecule or molecular association this osmotic pressure will naturally be small as compared with that produced by equal weight of a crystalloid.

THEORIES OF OSMOTIC PRESSURE

Explanations that have been put forward of the origin of osmotic pressure fall into two groups. In the first group osmotic pressure is supposed to arise in a similar way to gas pressure by the bombardment of the molecules (or ions) of the solute upon the separating membrane.

This theory may be called the kinetic theory of osmotic pressure. It was first put forward as a possible explanation by van't Hoff and is suggested at once by the fact that the osmotic pressure of dilute solutions is equal to the pressure the solute would exert as a gas occupying a volume equal to that of the solution. The contrasted hydrostatic theory, due in the first place to Jäger (1891 *a, b*) finds an origin for osmotic pressure in the different surface tensions of solution and pure solvent. This view, as elaborated by Moore (1894) regards

osmotic pressure as due to the movement of liquid through the capillary spaces of the semi-permeable membrane from the liquid of lower surface tension to that of higher surface tension. Modifications of the theory have been proposed by J. Traube (1904 *b*, *c*) and Battelli and Stefanini (1905 *a*, *b*, 1907 *a*, *b*, *c*) a presentation of which is outside the scope of the present work. For an adequate discussion of the views of these and other writers the reader is referred to Findlay's monograph already cited.

NEGATIVE OSMOSIS

In all the cases so far discussed in which a solution is separated from the solvent by a membrane, there results a passage of liquid across the membrane from solvent into solution. It is now necessary to turn to a phenomenon which appears anomalous and inexplicable by the laws so far considered. This is the phenomenon of *negative osmosis* characterised by the flow of liquid across the membrane from the solution to the pure solvent.

Negative osmosis was observed by Dutrochet (1827) and later by T. Graham (1854). The phenomenon has recently formed the subject of a series of investigations by Girard, working with membranes of pig's bladder, and by Bartell and his collaborators, who employed membranes of porcelain and gold-beaters' skin. The results of these investigations seem likely to have important bearings on questions of cell permeability, so that some detailed consideration of them appears necessary.

Bartell (1914) first worked with membranes of porcelain about 5 mm. thick, the pores of which were about 0.2μ in diameter. This diameter of pore is on the border-line as regards osmotic effects; with somewhat wider pores osmotic effects are not produced, while with narrower pores the osmotic effects are definite. The membranes are thus decidedly permeable to dissolved substances.

Osmotic cells were prepared in which the separating membrane was porcelain, and the cells were filled with solutions of the salts examined. These included chlorides, nitrates, sulphates and acetates of a number of monovalent and divalent kations in concentrations of $M/10$ and, in the case of sulphates, $M/5$ and $M/2$. In all the experiments in which potassium and ammonium salts were employed passage of liquid took place in the normal direction, that is, from the solvent to the solution (positive osmosis), with sodium sulphate and sodium acetate positive osmosis also occurred, as it did also in experiments with lithium sulphate; on the other hand, the chlorides and

nitrates of sodium, lithium, zinc, manganese and magnesium, the sulphates of the last three named metals, and the acetates of zinc and manganese all gave negative osmosis. Confirmatory results were obtained by using pure water in the cell and solutions outside.

It is important to observe that the membranes employed are not semi-permeable, and indeed the incidence of negative osmosis is related to the diameter of the pores in the membrane. Thus with $M/10$ magnesium chloride solutions, and with porcelain membranes, no apparent osmotic effects were produced when the diameter of the pores was greater than 0.4μ, with pore diameters between 0.4μ and 0.1μ negative osmosis was observed, while with pore diameters less than 0.1μ osmosis was in the positive direction as with semi-permeable membranes.

Further observations were made by Bartell and Hocker (1916 *b*) on the osmosis of the following solutions: (1) nitrates of a number of univalent (K, NH_4, Na, Li), bivalent (Ba, Zn, Mn, Mg), trivalent (Al) and tetravalent (Th) kations in concentrations varying from $0.0005\,M$ to M; (2) $0.1\,M$ solutions of a number of potassium salts (NO_3, Cl, Br, I, CNS, acetate, SO_4, CrO_4, PO_4, ferrocyanide and ferricyanide); (3) hydrochloric acid and sodium hydroxide in concentrations from $0.001\,M$ to $0.2\,M$, and (4) $0.1\,M$ solutions of some nitrates when the membranes were immersed in solutions of acids and alkalies in different concentrations.

The results obtained were very varied. In some cases positive osmosis occurred in all concentrations, increasing regularly with increase in concentration, in others positive osmosis occurred in low concentrations but decreased with increasing concentration so that in higher concentrations negative osmosis occurred. In other cases there was observed a definite concentration for maximum positive osmosis which decreases with either higher or lower concentrations, while with yet other electrolytes there is a concentration for maximum negative (or minimum positive) osmosis, all concentrations above or below this value giving more positive effects.

More recently Bartell and Madison (1920 *a*) have shown that abnormal osmosis also occurs with membranes of gold-beaters' skin, the flow of water across the membrane being sometimes in the direction of solvent to solution, sometimes in the reverse direction. In the case of normal (positive) osmosis, the flow, as compared with that into a solution of sucrose, was sometimes abnormally large and sometimes abnormally small. The same authors further show (1920 *b*)

that the osmosis of salt solutions is markedly affected by the presence of acids and alkalies.

The explanation of negative osmosis put forward by Girard (1908, 1909, 1910 *a*, 1910 *b*, 1914 *a*, *b*) and also by Bartell and his collaborators (1914, 1916 *a*, 1920 *a*, *b*) ascribes the phenomenon to the action of electrical forces. It has already been noted (Chapter III) that at the surface of separation of two liquids there exists a potential difference. But Bartell and Madison quote the results of Brünings (1903, 1907), Lillie (1911), Beutner (1913 *e*), Girard and Bartell and Hocker to show that permeable membranes of almost any material separating water and a solution exhibit a difference of potential on the two sides of the membrane which is different from the potential difference arising from contact of the two liquids. Girard supposes that in addition to the difference of potential between the two surfaces of the membrane there is also an electric charge on the walls of the capillaries of the membrane, while the liquid in the capillaries possesses a charge opposite to that on the walls of the capillaries, there being produced a Helmholz double layer (Helmholz, 1879). This charge on the walls of the capillaries is supposed to be determined by the preponderance of a small excess of hydrogen or hydroxyl ions as suggested by Perrin (1904). Such a state of affairs would tend to the movement of negatively charged water, for example, in the direction of the surface of the higher potential, and *vice versa*.

The explanation of negative osmosis offered by Bartell and his collaborators is essentially similar. The tendency to normal osmosis, determined by the differences in salt concentration on the two sides of the membrane will always be present, but superimposed on these is the passage of water brought about by the presence of electrical factors. These are as Girard suggested (1) the electric charge on the membrane with respect to the liquid layer in the capillaries of the membrane, that is, the value of the Helmholz double layer between membrane and liquid, and (2) the polarisation of the membrane, that is, the difference of potential between the two faces of the membrane. The value of the former we have noted is ascribed by Girard to the influence of an excess of hydrogen or hydroxyl ions. Bartell and his collaborators throw doubt on the adequacy of this explanation and suppose three factors to be active here, namely (*a*) the extent of the diffusion of the electrolyte through the membrane (phase potential), (*b*) the relative migration velocities of the ions (diffusion potential), and (*c*) the extent of selective adsorption

by the membrane (adsorption potential). These three ways in which potential differences may arise have been described in Chapter III.

There are nine possible cases of electrification, and these are clearly set out in diagrammatic form by Bartell and Madison. These are here shown in tabular form in Table XVI.

TABLE XVI

Possible Cases of Electrification of Membranes and
the Effect on Osmosis

No.	Charge on solution side of membrane	Charge on liquid in capillaries	Osmosis
1	None	None	Normal
2	None	+	Normal
3	None	−	Normal
4	+	None	Normal
5	+	+	Abnormally low
6	+	−	Abnormally high
7	−	None	Normal
8	−	+	Abnormally high
9	−	−	Abnormally low

It is to be observed that all these expected results have been realised in practice, as the summary of Bartell's work already given here has indicated. In cases 5 and 9, where we have respectively positively charged water moving to the side of the membrane negatively charged, and *vice versa*, the tendency for liquid to move from the solution into the solvent may exceed the tendency for normal osmosis to take place, in which case negative osmosis results. It is to be noted that both Girard (1919 *a, b, c*) and Bartell and Madison (1920) measured the potential difference between the two sides of the membranes they employed, and have shown that this is such as the theory requires. It is also possible that the electrification of the system might arise in other ways (cf. Freundlich, 1916). The phenomenon, it will be observed, is similar to that of electrical osmosis, in which water passes from one solution to another across a membrane as a result of a potential difference applied to the solutions on either side of the membrane. (See especially Briggs, 1919).

A curious case of negative osmosis has recently been noted by Loeb (1920 *a*). When solutions of aluminium chloride or of lanthanum salts are separated from water by a collodion membrane treated with gelatin, water diffuses into the solution, although such diffusion does not take place when an untreated membrane is used. If such

an untreated collodion membrane separates water and a solution of an acid in concentration less than $M/16$, positive osmosis occurs, the more rapidly the higher the valency of the anion. When, however, a similar membrane treated with gelatin is used, negative osmosis takes place. Similar results were observed with membranes treated with casein, edestin, egg-albumin and serum albumin, but not with peptone, alanine or starch. These results are attributed to electrical forces which in concentrations of solutions below $M/16$ mainly determine the osmotic effects. In passing through the untreated collodion membrane the water is positively charged; on treating the membrane with gelatin the charge on the water becomes negative, so that the electrical forces will tend to send the water in the opposite direction. Further evidence for, and elaboration of, this view is contained in a series of papers by Loeb (1920 *b, c, d, e, f, g,* 1921) to which further reference may be made. Enough has already been said here to indicate the importance in connection with osmosis of electrical phenomena associated with membranes. That such phenomena are present in the living animal cell has already been indicated by Girard (1919 *a, b, c*) and Girard and Morax (1920).

CHAPTER VII

THE CELL WALL

I T is characteristic of the plant cell as contrasted with the animal cell that the living protoplasts are surrounded by what is generally regarded as a non-living envelope, the cell wall. Certain plant cells, such as some unicellular algæ, spermatozoids and egg-cells are without this envelope, while some animal cells possess a very definite membrane surrounding them, such as the unicellular *Vorticella* and the eggs of some marine organisms.

But quite apart from the cell wall which forms an undoubted membrane surrounding the protoplast, it is the general opinion of botanists and physiologists that a membrane surrounds the protoplast, separating the general body of it from the cell wall. To this supposed membrane a variety of names have been given, of which external plasmatic membrane, plasma-membrane and ectoplast are those most usually employed. A similar membrane, the internal plasmatic membrane, tonoplast or vacuole wall is also recognised (de Vries, 1885) and there are even supposed to be similar membranes around the nucleus and all cell inclusions (see *e.g.* Vonwiller, 1918). On the other hand, some workers, principally those interested in animal cells, deny the presence of such membranes altogether. As the nature of the cell membranes is obviously a very fundamental matter in regard to cell permeability, some little space will here be devoted to a consideration of the cell wall, while in the next chapter consideration will be given to the evidence for the existence of plasmatic membranes.

STRUCTURE AND COMPOSITION OF THE CELL WALL

Cell walls exhibit a great range in composition and structure according to the age of the cell and the tissue of which the cell forms a part. The cell wall which arises between the daughter cells after the division of a meristematic cell is always at first thin and apparently homogeneous in structure, and remains thin so long as the cells it separates are meristematic. But as conversion of the cells into part of a permanent tissue takes place, the cell wall increases in thickness, the material added in this process being apparently deposited in at least two different ways, known as growth by apposition and growth by intussusception. In the former fresh particles are added on the surface of the existing wall. Where this method takes the form of the deposition of entire discernible layers or lamellæ it is sometimes distinguished as growth by superposition. In growth by intussusception the new particles are deposited within the existing cell wall, so that they form an intimate mixture with the pre-existing particles. The method of thickening the cell wall may play an important part in determining its permeability.

Stratification of the cell wall is stated to be almost universal (Haberlandt, 1914). It is clear that this appearance must be due to differences in composition of adjacent layers, the differences being either physical, such as differences in water content (Nägeli, 1866) or in chemical composition.

In all cases it appears possible to distinguish between a layer forming the middle of the cell wall, *the middle lamella*, and layers on each side of this. It has been stated that the middle lamella in soft tissues is composed of a calcium compound of pectin, a name given to a group of substances which are nearly related to pentosans, that is, condensation products of pentose sugars. A substance or group of substances described as pectin or pectinogen (Schryver and Haynes, 1916) can be isolated from the walls of plants, and this substance can be converted by a clotting enzyme pectase into an acid, pectic acid, with splitting off of methyl alcohol and acetone (cf. Tutin, 1921). This acid forms soluble salts with the alkali metals, but an insoluble salt with calcium, and it is this salt which it has been supposed forms the middle lamella of parenchymatous cell walls. However, if plant tissue is treated so that all the pectin compounds, including the calcium pectate, are converted into pectic acid, and is then treated with a solution of ammonium oxalate or dilute sodium or ammonium hydroxide, the pectic acid dissolves, so that cells

separated by a pectic middle lamella fall apart. Such a maceration of tissue certainly takes place in many cases, but in many other cases it does not. Thus Professor Priestley informs me that in a bean stem after such treatment the epidermis, endodermis and cambium still remain as continuous layers, from which it is to be concluded that the radial walls of these cells do not possess a pectic middle layer. Professor Priestley also finds that the cells at meristematic apices of roots do not possess a pectic middle lamella, a very important finding.

The bulk of the cell walls in soft tissues is composed of cellulose, a group of substances which on hydrolysis yield glucose, and which are therefore to be regarded as glucosans. In addition to cellulose, or rather, celluloses, there may also be substances representing intermediate stages between cellulose and glucose, to which the substances termed "amyloid" appear to belong. Condensation products of other sugars such as mannose and galactose (mannosans and galactosans), included in the inappropriate term "hemicelluloses," may also be present, in addition to pectin. These substances are all complex carbohydrates, for information with regard to which reference may be made to standard chemical textbooks of the subject (*e.g.* Tollens, 1914; Haas and Hill, 1920). In these so-called cellulose walls other organic substances are also present. Thus Hansteen-Cranner (1914) has extracted fatty substances from the cell walls of the young parts of *Ricinus communis*, *Vicia Faba*, *Cucurbita Pepo* and a number of other species.

Apart from these organic constituents, the cell wall often contains a certain amount of mineral matter, silica, calcium oxide and calcium carbonate being the inorganic substances most frequently met with.

It is important for our purpose to know how these various substances found in the cell wall are there related to one another. It is clearly a feasible view to suppose the cellulose forming a solid framework, in the meshes of which the pectin and lipoid and other substances constitute the disperse phase of a hydrosol or hydrogel. This view, to which the writer inclines, would account for the rigidity and definitely solid character of the cell wall, and at the same time account for its powers of imbibition. But there are others who regard the cellulose itself as also forming part of the colloidal system. Thus Hansteen-Cranner, who thinks the fatty substances found by him in the cell wall are there probably in the form of salts of fatty acids, that is, as soaps, regards the cell wall as a hydrogel complex of which the more solid phase is composed of the colloid cellulose + pectin + colloidal soap. He was indeed able to prepare a compound of pectin and lauric acid and to manufacture membranes of this substance.

Cellulose walls are generally regarded as completely permeable both to water and the vast majority of dissolved substances, the chief evidence in support of this opinion being derived from the phenomenon of plasmolysis. This is the well-known contraction of the protoplast from the cell wall when the cell is immersed in a solution of higher osmotic concentration than that of the cell sap. Were the cell wall permeable to water but not to the dissolved substance in the external solution, the cell wall would constitute a semipermeable membrane and so would be the contracting membrane to which the protoplast would continue to adhere.

However, if we accept the view which appears inevitable, having regard to the chemical composition of the cell wall, that the latter is, at least in part, a colloidal system, there appears every reason to suppose that the cell wall may play a part in determining the interchange of substances between the cell and its exterior. Thus Devaux (1901) showed by a spectroscopic method that the cell walls of a number of different species from different groups of the plant kingdom possess a strong absorptive capacity so that not only the ions of heavy metals such as iron, copper, silver and lead are absorbed, but also those of the alkalies and alkaline earths. A metal absorbed in the cell wall can be displaced by another. It will be recalled that relatively more substance is adsorbed from a weak solution than from a strong one, other conditions being equal, and Devaux has shown that absorption by the cell wall takes place from very dilute solutions. This author (1904) suggested that the absorption of substances by the thin walls of the root hairs facilitates the bringing of these substances into contact with the protoplasm of the root hair. Since Devaux treated his material with eau de Javelle, weak acid and pure water, Hansteen-Cranner points out that he must have removed the fatty acids and soaps from the cell wall, so that actually the absorptive capacity of the wall must be considerably greater than indicated by Devaux's results, for the lipoid substances he removed are known to possess strong adsorptive properties. The assumption that the phenomena observed by Devaux are due to adsorption (cf. Czapek, 1913; Hansteen-Cranner, 1914) appears not altogether justified by the facts; they might be explained as due to chemical action.

In a series of experiments with parchment membranes and with membranes prepared artificially from cell walls, Hansteen-Cranner has shown that the amount of water absorbed by a cell wall membrane in unit time is influenced by the composition of the solution in which

the membrane is immersed. The membrane takes up less water from a $0·02 M$ solution of calcium chloride than from solutions of sodium and potassium chlorides of the same molecular concentration. Membranes from which the lipoid substance had been removed took up more water than similar membranes containing fatty constituents. Parchment membranes did not resemble cell wall membranes with regard to the influence of the composition of the surrounding solution on their uptake of water.

It is clear from these considerations that the cell wall cannot be dismissed as a negligible factor in cell permeability.

We have so far considered the cell wall of young active cells in which thickening has not taken place, or in which the thickening consists of "cellulose." But in many cases the cellulose walls become modified so that their permeability properties undergo much alteration. In some cases the change results in the wall becoming mucilaginous. It appears that in some species at any rate, the mucilage results from the conversion of polysaccharides into pentosans (condensation products of pentose sugars) which have great water-absorbing capacity (MacDougal, Richards and Spoehr, 1919). Other cell walls become lignified, suberised or cutinised (cuticularised). These changes are due to the deposition in the cell wall of substances, or rather mixtures of substances, known as lignin, suberin and cutin respectively. Among the substances which compose lignin a number have been isolated which are claimed to be characteristic of lignified walls; among these are xylan, lignic acids, and hadromal, an aromatic aldehyde (Czapek, 1899). Particularly important from the point of view of the student of permeability is the fact that in lignified walls the "cellulose" and lignin appear to be present in an intimate mixture (Robinson, 1920). This may partly account for the fact that water passes readily through lignified walls, but not through suberised walls.

Suberin is the name given to the group of substances to which the characteristic properties of cork are due. Various substances have been isolated from suberin, among these being the so-called suberogenic acids (Gilson, 1890). Some of these substances have been obtained pure and empirical formulæ obtained for them, *e.g.* phellonic acid, $C_{22}H_{43}O_3$, and phloionic acid, $C_{11}H_{21}O_4$ (after drying for weeks, $C_{20}H_{40}O_7$), while a third substance, suberinic acid, appears to possess the formula $C_{17}H_{30}O_3$. There appears to be a divergence of opinion on the fatty nature of suberin, but Gilson decides that the substances composing it cannot be regarded as true fats, an opinion with which Priestley (1921) agrees.

.   The significance of these suberogenic acids in regard to the impermeability to water of suberised walls is indicated by an experiment described by Priestley in which a Soxhlet thimble impregnated with a condensation product of potassium phellonate was found to be completely impermeable to water. It is significant in this regard also that according to van Wisselingh (1886–1892) the middle lamella of suberised walls consists of an unbroken layer of suberin, so that although cellulose is subsequently deposited on suberised walls, there is always present this continuous layer impermeable to water separating the cork cell from adjoining cells.

Cutin forms a continuous layer on the outside of the outer walls of the cells of leaves and herbaceous stems. Like suberin it is impermeable to water, and it is probably also like suberin in consisting of a mixture of several substances, although the evidence is not so definite. The cutinogenic acids obtained from cutin are, however, not the same as the suberogenic acids obtained from suberin.

With the chemistry of lignin, suberin and cutin we cannot deal further here. Those interested should consult the standard textbooks on the subject (especially Czapek, 1913) and the recent paper by Priestley already cited.

The coats of many seeds have a very low permeability to water. In some cases this is no doubt due to cutinisation or suberisation of cell walls, but in other cases to the presence of tannins, lipoid and pectic substances, as treatment of certain seed coats (*e.g.* pumpkin, almond, peanut) with solvents for these substances greatly increases the permeability (Denny, 1917 *b*).

Before leaving the subject of the cell wall, it should be noted that the young cell wall is capable of stretching when acted upon by a force, while on removal of the force it tends to return to its original condition. It is thus elastic. With change in composition as the wall gets older this extensibility and elasticity may be lost, but cellulose walls generally retain extensibility and elasticity even when considerably thickened.

Mention has already been made of the pits in cell walls (cf. Chapter II). As the rate of diffusion through a membrane when diffusion is proceeding at a uniform rate varies inversely as the thickness of the membrane, it has been supposed that these pits help to expedite diffusion (Haberlandt, 1914) as the pit-closing membrane is considerably thinner than the thickened part of the cell wall. It is to be supposed in this connection that the relation between diameter of the pore and rate of diffusion through the pore found by H. Brown

and Escombe (1900) also holds for the diffusion of substances in solution, and that as the diameter of the pit is small the rate of diffusion through it is proportional to the diameter and not to the area, of the pit, so that with decreasing area of the pit, the rate of diffusion decreases much more slowly. Similarly, the law for diffusion through a multi-perforate septum may also be presumed to hold.

The presence of chitin in the cell walls of Fungi has already been noted. For a summary of the literature dealing with the composition of the cell walls of these and other lower plants, the walls of which exhibit many differences from those of the higher plants, reference should be made to the first volume of Czapek's *Biochemie der Pflanzen* (1913), and for a description of the cell wall of some red algæ a paper by Sauvageau (1920) may be consulted.

SEMI-PERMEABLE CELL WALLS

Besides cell walls which allow the passage of water and most dissolved substances, and cutinised and suberised walls which are practically impermeable to water, there exist cell walls which are readily permeable to water, but impermeable, or almost so, to such simple solutes as sodium chloride (Gola, 1905). Such membranes were noted by A. J. Brown (1907) in the grain of barley. Barley grains readily absorb water from aqueous solutions of sulphuric acid, hydrochloric acid, cupric sulphate, ferrous sulphate, potassium chromate, silver nitrate and potassium ferrocyanide, while chemical analysis of the solutions shows that the dissolved substance is not absorbed. It thus appears that there is a semi-permeable membrane enclosing the seed of barley. That this membrane is not a living protoplasmic one becomes clear from the fact that grains can exhibit the semi-permeable action in strong solutions of such poisons as cupric sulphate and silver nitrate, which would inevitably destroy the protoplasm and its semi-permeable properties if they came into contact with it, while conclusive proof is obtained from the fact that grains killed by boiling still exhibit the property.

It is a curious fact that iodine, in solution in potassium iodide, is able to penetrate the membrane without apparently injuring it, for the subsequent passage of sodium thiosulphate is still prevented. Nitric acid, on the other hand, appears to destroy the membrane, for this acid is excluded for a time, but subsequently enters the grain.

Further evidence of the presence of a semi-permeable membrane in the grain of barley is given by the fact that the blue pigment contained in the aleurone cells of the grain of *Hordeum vulgare*, var.

cærulescens, undergoes no change in colour when steeped in a solution of sulphuric acid, while in the presence of acid the pigment changes in colour to red.

In a subsequent paper A. J. Brown (1909) showed that less water is absorbed by barley grains from salt solutions than from pure water, a result to be expected if the covering of the grain contains a semi-permeable layer (compare Chapters VI and VIII).

Such semi-permeable membranes formed by cell walls have been shown by A. J. Brown (1907) to be present also in grains of *Avena*, *Secale* and *Triticum*, the semi-permeable membrane of the last named having also been investigated by Schroeder (1911). Gassner (1915) also records the presence of semi-permeable cell layers in seeds of Gramineae, while they have been observed by Tjebbes (1912) in sugar-beet seed, by Shull (1913) in the seeds already mentioned and in apple, pear, *Vicia Faba* and other Leguminosae, *Helianthus annuus*, *Xanthium glabratum* and *Alisma plantago-aquatica*, and by Rippel (1918) particularly in *Æsculus hippocastanum* and to a less extent in a number of other species. Rippel soaked seeds in a solution of $N/10$ sodium chloride, measured the uptake of water by weighing the seeds, and the intake of sodium chloride by titrating the solution against silver nitrate. He showed that generally speaking the greater the intake of water, the greater the intake of salt. His results are summarised in Table XVII.

TABLE XVII

Relation of Semi-permeability of Seeds of Various Species to Water Intake (from Rippel)

Species	Water uptake %	Ratio of concentration of external solution to the theoretical concentration at the end of the experiment if the seeds are completely semi-permeable
Trifolium pratense	141·2	56·6
Sinapus alba	118·0	64·3
Phaseolus multiflorus	106·4	90·0
Pisum sativum I	87·4	82·5
,, ,, II	83·0	85·9
Vicia Faba	65·2	91·3
Lychnis Githago	60·0	98·4
Rye	48·5	94·3
Cannabis sativa	48·2	95·8
Æsculus hippocastanum	21·8	100·0

Rippel suggests that these results may be explained on the view that the swelling of the seed by absorption of water ruptures the semi-permeable membrane.

With regard to the position of the semi-permeable layer very diverse opinions have been expressed. From the experiments with *Hordeum vulgare*, var. *cærulescens*, and by following the penetration of sodium chloride by silver nitrate, A. J. Brown thought it possible that the walls of cells derived from the nucellus form the semi-permeable membrane in barley grains. Schroeder in the case of wheat suggested the possibility of a localised entrance of water and dissolved substances into the grain, or possibly decreasing permeability of the coverings from the basal to the apical end of the grain. Reichard (1909) correlated the presence of a tannin layer in the barley grain with semi-permeability. Shull (1913), from his investigations on a number of species, particularly *Xanthium glabratum*, concluded that cellulose walls may form semi-permeable membranes, and stated that "the possible semipermeable character of cellulose membranes cannot be overlooked in future investigations dealing with the entrance of salts into plant tissues."

Shull disposed of Reichard's view that tannin determines the semi-permeable character of a layer in the seed coats of Gramineae by extracting the tannin from grain with sodium hydroxide without affecting the semi-permeable properties, while Collins (1918) was unable to confirm the presence of a tannin layer in barley grains.

The question appears to have been solved for the case of the barley grain by Collins who, in a careful morphological and physiological investigation, showed that the barley grain is completely surrounded by a strongly cutinised membrane except in the region of the micropyle and the chalaza. Only a very small proportion of the water absorbed by the grain can pass the cutinised layers, while dissolved substances are also kept back. The seat of rapid entry of water is at the micropylar region of the grain, and here must be the seat of semi-permeability. Entry of water through the chalaza appears much slower. The cells in the micropylar region (the so-called embryonic appendage) through which water must pass, appear possibly to be modified in the direction of pectic, mucilaginous, or gum compounds. The conclusion is not definite, and so cannot be used as evidence either for or against Shull's opinion of the possible wide distribution of semi-permeable cellulose membranes in plants.

From this review of the properties of cell walls with regard to permeability it is clear that we may find walls with very different degrees of permeability both to water and to various dissolved substances.

CHAPTER VIII

THE PLASMA-MEMBRANE

THE presence of a layer of protoplasmic material limiting the general body of the cytoplasm and differing from it in permeability properties, is regarded by many botanists as essential for explaining the relation of the cell to water and its differential behaviour towards dissolved substances in regard to their absorption. Such membranes are, however, not morphologically obvious in most cells, and some investigators dealing principally with animal cells, have denied the existence of such protoplasmic membranes altogether. An enquiry is obviously necessary here into the evidence for the existence of such limiting membranes.

The question has not been simplified by a somewhat confused terminology. By the term plasmatic membrane or plasma-membrane Pfeffer (1877, 1890) clearly meant to denote a surface layer of the protoplasm with semi-permeable properties, but the character of which was not further defined. De Vries (1884 a, 1885) used the terms ectoplast and tonoplast for layers limiting the protoplasm externally in contact with the cell wall and internally in contact with the vacuole respectively. These terms were used by de Vries and later by Livingston (1903) so as to imply apparently the same as Pfeffer's term plasmatic membrane.

Again, in some cells, as for example in the plasmodia of Myxomycetes, there is a peripheral layer of the protoplast morphologically distinct from the internal mass of the protoplasm, in that under the microscope it appears clear and not granular. It is not to be assumed that this outer layer, the hyaloplasm, is identical with the plasma-membrane of Pfeffer. "A hyaloplasmic border of measurable thickness can hardly be identical with the plasmatic membrane throughout its whole extent, although the mere existence of such a border, since it indicates that its internal surface has the power of repelling granules, shows that a certain similarity must exist between its characters and those of a plasmatic membrane" (Pfeffer, 1900). The ectosarc of *Amœba* and the ectoplasm of some plant and animal cells appear to be similar to the hyaloplasm.

For the sake of clearness therefore, the term plasma-membrane will be used to denote a surface layer of protoplasm which behaves as a membrane surrounding the bulk of the protoplasm, and which , may exhibit different degrees of permeability to different substances, readily permitting the passage of some, less readily permeable to others, and to yet other substances quite impermeable. The membrane bounding the outside of the protoplast, and so in contact with the cell wall, will be called the external plasma-membrane, and that bounding the vacuole, the internal plasma-membrane. For the visibly distinct outer layer of the protoplasm the term hyaloplasm will be used. Of the existence of this in certain cells there is no doubt. What we are immediately concerned with here is the existence of the plasma-membrane. Should the evidence for the existence of the plasma-membrane be regarded as convincing, the question then arises of the identity of the plasma-membrane with the hyaloplasm.

There are five main lines of evidence for the existence of plasma-membranes. These will now be considered.

1. Evidence from the Physical Laws of Surfaces

Surface phenomena have been discussed in some detail in Chapter III, where it has been pointed out that the surface of separation between two phases, such as we have at the boundary between the protoplast and the cell wall, and between the protoplast and the vacuole, has physical properties different from those of the bulk of either phase. It has been noted that one result of these different physical properties is that any substance present in solution which lowers the surface tension of the solvent will tend to accumulate at the surface. Hence substances present in the protoplasm which lower the surface tension of water will be present in the surface layer in higher concentration than in the bulk of the protoplasm. That the protoplasm contains many such substances there can be no doubt, and the presence of these substances in greater concentration in the surface layer than in the internal mass of the cytoplasm, might in itself account for a difference in permeability properties between the surface and the interior of the cytoplasm. But the cell wall and the vacuole no doubt also contain substances which lower the surface tension of water, and these will also tend to accumulate at the surface of the cell wall and vacuole respectively. Thus at the interfaces, cell wall-protoplast and protoplast-vacuole, there must be present thin layers of material which differ both physically and

chemically from the phases which they separate. These layers are no doubt largely composed of loosely bound adsorption compounds, but it is always possible that the meeting of substances adsorbed from the one and the other of the two phases in contact may result in the formation of more stable chemical compounds which are not present in the interior of either of the two adjoining phases. In such a case the plasma-membrane would be regarded as produced in much the same way as the precipitation membranes discussed in Chapter V. The formation of a rigid film at the surface of separation of a protein solution and some other phase (Ramsden, 1903), presumably by the protein adsorbed at the surface undergoing some irreversible change, is of significance in this connection.

In any case it is clear that from purely physical and physico-chemical considerations there is good reason to suppose that where the protoplast is in contact with the cell wall and the vacuole there are thin layers which differ in physical and chemical properties from the general mass of the protoplasm. It is possible and, indeed, to be expected, that among other properties in which these layers differ from the internal cytoplasm, is their permeability to different substances.

2. EVIDENCE FROM CYTOLOGICAL OBSERVATIONS

It is convenient to group under this second heading all the evidence bearing on the existence of plasma-membranes which is based on actual observations made on the protoplasm of the cell. Such observations are of three kinds: (1) ordinary microscopic observations, (2) ultramicroscopic observations, and (3) those made by the aid of microdissection.

(1) *Microscopic observations.* Incidental reference has already been made to the clear hyaloplasmic layer of Myxomycetes and of the cells of other organisms. The hyaloplasm is presumably more viscous than the internal mass of the cytoplasm as it appears free from moving granules which would thus seem to be repelled from entering it. It is to be noted that the existence of the hyaloplasm appears to be due to surface forces, for it remains constant in thickness when the cell increases or diminishes in volume owing to absorption or loss of water (Bayliss, 1915).

Sometimes in cells that have been killed a very fine membrane can be recognised limiting the now dead protoplasm. As Bayliss (1915) points out, this "is no proof that the membrane was in existence in the living cell in the same state as that seen."

The method of de Vries (1885) of rendering visible the inner plasmatic membrane, by plasmolysing with 10 per cent. potassium nitrate and staining with eosin, is open to similar objection.

In experiments on the penetration of a number of dyes into intact cells of a number of plant and animal species, and through the internal protoplasm of torn cells, Kite (1913 *a*, 1913 *b*, 1915) finds the surface layer of the cells he investigated is generally more easily penetrated by a dye than the interior protoplasm. So far, however, from regarding this as any evidence of the existence of a limiting plasmatic membrane, he speaks of such membranes as hypothetical structures. Although his results are in direct contradiction to the general opinion of the permeability properties of plasmatic membranes, it is not clear how he comes to regard such membranes as non-existent.

(2) *Ultramicroscopic observations.* The observations of Gaidukov and Price with dark-ground illumination have been referred to in Chapter II. Some of their observations relate to the plasma-membrane.

Gaidukov (1910) records a differentiation between the outer layer of the cytoplasm and the internal part of the cell, but it is not clear from his description whether this outer layer can be referred to hyaloplasm or not. The observations of Price (1914) are more definite. In the cells of the leaf of *Elodea canadensis* "no definite differentiation into endoplasm and ectoplasm was made out." In the cells of the large multicellular hairs of the stems and leaves of *Cucurbita* spp. "there is what appears to be a slight membrane limiting the protoplast towards the vacuole, and also forming the surfaces of the strands" (that is, of protoplasm). In the cells of the hairs of the tomato an outer layer of the cytoplasm appears to be differentiated from the rest of the cytoplasm, the particles in Brownian movement being smaller and less numerous than in the main mass of the protoplasm. In a series of observations made on the germination of spores of a species of *Mucor*, the contents of the ungerminated spore appeared almost homogeneous, but after absorption of water had taken place for several hours a peripheral layer with the appearance usually attributed to a gel became differentiated from the general mass.

Price also studied the appearance of plasmolysed cells under dark ground illumination. He states that in *Spirogyra* plasmolysed with dilute glycerol a layering of the protoplast can be observed, an external layer containing extremely fine particles becoming differentiated from the inner region containing the larger microsomes.

The outer layer appears to be drawn out in places into threads which at their outer end retain contact with the cell wall. Such fibrils have frequently been recorded as occurring in plasmolysis (Gardiner, 1884; Bower, 1885; Chodat and Bourbier, 1898; Hecht, 1912). The particles in the outer region were in active movement, and there was no sign of a hydrogel layer limiting the protoplast, but Price suggests that some definite boundary must be present as the colloid particles do not escape into the surrounding liquid. Cells plasmolysed with 10 per cent. potassium nitrate presented a similar appearance.

Sometimes during the plasmolysis of *Spirogyra*, vesicles of protoplasm containing presumably cell sap are formed (cf. de Vries, 1885). The wall of the vesicle contains particles in active movement and if any part of the protoplasm is in the gel state it must be an extremely thin layer, for none is obvious. In *Mougeotia* on the contrary, the wall of the vesicle appears to be rather in the gel condition.

During plasmolysis of the cells of the hairs of *Cucurbita* the protoplast appears to be bordered on the outside by a definite transparent membrane on which finer particles in the interior of the protoplast appear to impinge. This membrane was observed when plasmolysis was brought about by 30 per cent. sucrose, and so is presumably to be regarded as normal and not produced by the coagulating action of electrolytes.

It is scarcely possible to draw any very definite conclusion from this work with regard to the general presence of a plasma-membrane. In the hairs of the tomato the outer layer differentiated from the rest of the cell contents appears to be identical with hyaloplasm; in the hairs of *Cucurbita* there appears to be a thinner layer of a gel nature, limiting the protoplast on the outside.

(3) *Evidence from the results of microdissection.* The observations made on the structure of protoplasm by means of microdissection have already been mentioned in Chapter II. The conclusions to which workers with this method come in regard to the plasma-membrane will now be indicated.

Chambers (1917) describes the peripheral layer of the cytoplasm of young germ cells, egg cells and Protozoa as much denser than the interior cytoplasmic sol, but merging insensibly into it, and concludes that this surface layer is a highly extensile, contractile and viscous gel. If this surface layer is injured a new and similar layer will form at the damaged place, the capacity for the establishment and maintenance of the viscous gel layer being a property essential to cytoplasm. The rate of diffusion of three dyes, neutral red, cresyl blue and janus

green, appeared to be the same in the surface layer as in the general body of the cytoplasm. In adult somatic cells, where the general mass of the cytoplasm is in the gel state, it is not possible to distinguish a differentiated outer layer.

The peripheral layer of gel observed by Chambers in young germ cells, egg cells and Protozoa, appears to be the hyaloplasmic border, and in the case of the Protozoa it is definitely spoken of as the ectoplast. Seifriz (1921) has, however, recently made a special study of protoplasmic membranes by the microdissection method, and concludes that in *Amœba* and the plasmodium of Myxomycetes the hyaloplasmic border is not to be regarded as the plasma-membrane, for there is evidence of a definite, though exceedingly delicate membrane limiting the hyaloplasm on its outer side. It is shown that the surface of a young and active pseudopodium is liquid, for if the hyaloplasm is pierced by a microdissection needle and this moved towards the edge of the protoplasm, the hyaloplasm will follow the needle so as to form an artificial pseudopodium, while on releasing the needle there is no appreciable contraction. This behaviour is only explicable on the view that the surface layer is liquid. But after the pseudopodium has advanced to a certain point, there is no further advance, to explain which it is supposed that a sudden change in consistency of the protoplasm from sol to gel takes place. This change must be at the surface, for the hyaloplasm changes little in viscosity. The surface layer of gel is now capable of a little stretching. This is the condition of a quiescent plasmodium. Seifriz thus concludes that "the inactive surface layer is a highly viscous emulsion colloid, undoubtedly in the gel state, which solates (*i.e.* becomes a sol) when streaming takes place, and reverts to the gel state when the plasmodium again becomes inactive. This surface layer is exceedingly delicate, of immensurable thickness, and is not identical with the hyaloplasmic border (or ectoplasm), which, to be sure, it resembles in constitution, but from which it is more or less sharply delimited." The state of affairs in *Amœba* is similar except that the hyaloplasm (ectoplasm) behaves in the same way as the membrane, though in the passive condition it is less rigid than the membrane and so can be distinguished from it.

From the fact of the regeneration of the plasma-membrane from cytoplasm containing hyaloplasm, it is concluded that the membrane is formed from the latter and is certainly not to be regarded as an autonomous organ as de Vries (1885) supposed.

In dead material a delicate membrane can sometimes be observed

surrounding the protoplasm. This is regarded by Seifriz as the plasma-membrane after it has become changed by "fixing" or coagulation. The occurrence of this "degenerate" membrane is cited by Seifriz as further evidence for the existence of the plasma-membrane. There is similar evidence for the existence of an internal plasma-membrane surrounding the vacuole, and also one separating the protoplasm and nucleus (the nuclear membrane).

It is to be noted particularly that the plasma-membrane is capable of undergoing reversible change from sol to gel, of readily adjusting itself to changes of shape and area, and if destroyed is in most cases readily and immediately re-formed at the surface of the cytoplasm, from which it is not capable of isolation until the cytoplasm has undergone degeneration. It is obviously to be regarded as part of the protoplasm.

3. EVIDENCE DERIVED FROM A CONSIDERATION OF THE WATER RELATIONS OF THE CELL

The simplest case that can be considered in this connection is that of a cell unprovided with a cell wall and in which vacuoles are not present. Such cells, common in animals, are comparatively unusual in plants. Animal cells are often surrounded by a solution of a number of salts, and although the concentration of this solution varies in different cases, the salts present in it are very generally those of sea water, and the relative proportion of the salts may be the same as in that medium. If cells, for example, red blood corpuscles, or pieces of tissue such as muscle or sheep's eyes, are placed in distilled or tap water, the cells absorb water, and this absorption may continue until the swelling is such that the cell or tissue is ruptured. The cells are thus behaving as if they consisted of a solution possessing an osmotic pressure and surrounded by a semi-permeable membrane. If this be the case, as the osmotic pressure exerted by the colloids of the cell must be very small, while the movement of water into the cell indicates a very considerable osmotic pressure, the existence of the latter must be due to crystalloids.

This is the usually accepted explanation of the swelling of cells in distilled water or in weak solutions, and of the corresponding shrinkage of cells in solutions of high osmotic pressure. It involves the assumption of a semi-permeable membrane enclosing the general body of the protoplasm. As Loeb (1906) has truly pointed out, it is not necessary that the membrane should be impermeable to salts for the absorption of water from a solution of such salts to take place,

for if the crystalloids only diffuse slowly into the protoplasm, while the membrane offers very little opposition to the passage of water, absorption of water will take place readily at first.

In young plant cells vacuoles are either non-existent or very small, and the system thus approaches very closely to such a cell as that considered above, but surrounded by a wall. The behaviour of such a cell towards water is similar to that already described, but the presence of the wall is responsible for certain differences. Thus, when the walled cell is immersed in water or in a dilute solution, water is absorbed, but this cannot proceed as rapidly or to the same extent as in the case of the unwalled cell, for as water passes into the cell the increase in volume involves a stretching of the cell wall which thus exerts a continually increasing tension inwards, so that on account of this water tends to be forced out from the cell. When these two forces, that forcing water in and that forcing it out, equal one another, an equilibrium condition is reached, and the protoplast is thus prevented from absorbing water until it bursts. With thin cell walls this can however sometimes happen. Thus some marine algæ, notably *Derbesia* and *Bryopsis*, when transferred to fresh water (Noll, 1888), will burst very easily. Lidforss (1896) has recorded the bursting of pollen grains of a number of species, notably those belonging to the Liliaceæ, in the same way. F. E. Lloyd (1915, 1916, 1917 *a*, 1917 *b*) has observed the bursting of pollen tubes of *Lupinus*, *Lathyrus* and *Phaseolus* in distilled water or weak sucrose solutions. The same author has even observed the bursting of pollen grains of *Gossypium* in 50 per cent. glycerol, 25 per cent. sucrose and 0·45 N potassium nitrate, surprisingly high concentrations. The tips of the hyphæ of moulds (Curtis, 1900) and the asci in some Ascomycetes (Livingston, 1903) will burst if placed in distilled water or in a very weak solution.

The water relations of the non-vacuolated cell can thus be easily explained if it is assumed that the outer layer of the protoplast consists of a membrane which is readily permeable to water but impermeable, or only comparatively slightly permeable, to many soluble substances present in the protoplasm. Such a view, as has already been stated, has its opponents, one of the most ingenious of whom, M. H. Fischer, has attempted to explain the absorption of water by organic tissue on account of the colloidal nature of the tissue. In a long series of experiments Fischer (1910, 1915) has shown that the swelling of certain proteins, namely gelatin and blood fibrin, in water and in solutions of acids, salts and alkalies, closely resembles

the swelling of tissues as exemplified by frog's muscle and sheep's
eyes.

Miss D. J. Lloyd (1916) has also made a study of the swelling of
excised sterno-cutaneous muscle of the frog in solutions of acids,
alkalies and salts. She comes to the conclusion that the swelling of
muscle both inside and outside the body is an osmotic phenomenon,
and compares her results with those of Lillie (1907), who showed
that acids and alkalies bring about an increase in the osmotic pressure
of gelatine and egg-albumin. Miss Lloyd accepts the view that acids
combine with colloids to give ionisable complexes which dissociate
into a large "pseudo-ion" carrying a positive charge and a mobile
ion with a negative charge; the degree of dispersity of the system is
increased and so the osmotic pressure is raised (cf. Hardy, 1900).
Similar considerations hold with regard to the action of alkalies. Miss
Lloyd thus agrees with Procter (1914) in ascribing to ionisation the
production of the internal osmotic pressure of such colloids, but
differs from him in supposing that the colloidal ion is partly responsible
for the osmotic pressure and not merely the mobile ion. In solutions
of neutral salts and in sugar solutions (for the latter see Hardy, 1905),
muscle may show a preliminary gain in weight, but sooner or later
coagulation sets in and loss in weight supervenes (cf. Lillie's results
with gelatin, 1907). Miss Lloyd ascribes this to the suppression of
the ionisation of the colloid particles of the muscle, which lose their
charge and are coagulated.

Miss Lloyd holds that there is no need to postulate the presence
of a semi-permeable membrane surrounding the muscle fibres, the
structure of the colloid in itself being presumably such as to prevent
the movement of the colloidal ions and consequently also of the
oppositely charged mobile ions.

Loeb (1916) has suggested a causal link between the development
of acidity in living muscle cells and the imbibition of water. It is
suggested that the acid brings about a hydration of the proteins to
such an extent that the true solutes are now held in a very reduced
volume of water. This marked rise in osmotic pressure brings about
the entrance of water into the cell.

The observations of F. E. Lloyd (1915, 1916, 1917 *a*, 1917 *b*) on
the effect of acids and alkalies on the growth of pollen tubes are of
particular interest in the present connection. Lloyd grew pollen
tubes in hanging drops of a solution of sucrose to which had been
added a definite quantity of acid or alkali and made observations on
the rate of growth of the tubes. In pure sucrose solutions the rate

of growth of the tubes was found to be inversely proportional to the concentration of sucrose, but if the concentration of the sugar was too low, the pollen tubes burst (see above). Thus the greatest rate of continued growth occurred in a solution of about 20 per cent. sugar. Since growth depends on the swelling of the protoplasm, that is, on the capacity of the protoplast to absorb water from the external medium, the introduction of substances into the external medium which alter the imbibition capacity of the protoplast differently from the osmotic pressure should give a means of determining whether the absorption of water by the cell is due to imbibition by the protoplast on account of its colloidal nature, or on account of differences in osmotic pressure on two sides of a semi-permeable membrane.

Lloyd found that acids (hydrochloric, nitric, acetic and citric) added to 20 per cent. sucrose, did not affect the growth rate when the concentration of acid was between $N/51200$ and $N/3200$ but that in higher concentrations, from $N/1600$ to $N/200$ the growth rates were less and less with progressive increase in the concentration and always terminated by bursting. When, however, acetic acid was added to 40 per cent. sucrose the growth rate when the acid was present in a concentration of $N/3200$ was increased to about four times that of the control. With alkalies added to 20 per cent. sucrose growth was depressed when the alkali was present in a concentration of $N/400$, but was greater when the concentration of alkali was within the range $N/800$ to $N/12800$, the greatest rates being with alkali concentrations of about $N/1600$ and $N/3200$. At the higher concentrations bursting of the tubes took place.

It will be noticed that the concentrations of acid and alkali used are negligible in comparison with that of sugar employed and so will not exert any appreciable influence on the value of the osmotic pressure of the external solution. On the other hand, the observations of many observers (for example, Pascheles, 1897; Wo. Ostwald, 1905, 1906; M. H. Fischer, 1910; Procter, 1911, 1914; Loeb, 1918) have shown that the swelling of gelatine and other colloids is greatly influenced by the hydrogen-ion concentration of the external medium. Lloyd has himself made experiments on the influence of acidity and alkalinity on the rate of swelling of gelatin. He concludes that for all acids there is a concentration which induces a maximum rate of swelling and a concentration which depresses swelling to a rate less than that of pure water. Alkalies produce a similar effect but it is not yet certain whether the minimum swelling rates are less than those occurring with distilled water.

From these experiments Lloyd thus comes to the conclusion that imbibition by the protoplasm rather than osmotic pressure is the dominant factor in growth.

While in general the water relations of the non-vacuolated cell can be explained by assuming the presence of a semi-permeable layer limiting the protoplast, there is yet a considerable body of evidence and opinion indicating the importance of imbibition by the colloidal protoplasm in determining the uptake and excretion of water by the living cell. Yet it has been shown that change in volume of cells of the kidney (Siebeck, 1912) and muscle cells (Beutner, 1912 a) placed in solutions of a substance which does not enter the cell are those which are to be expected if the protoplast is surrounded by a semi-permeable membrane. If the loss or gain in water by the cells were related solely to the imbibition properties of the protoplasm, a quite different relation should hold.

The evidence in regard to the presence of a semi-permeable membrane limiting the protoplasm of the non-vacuolated cell is thus rather conflicting. The observations at present available are much too scanty for us to draw any definite conclusions. There seems, however, to be definite evidence that in some cases at any rate, imbibition by the protoplasm plays a very definite part in determining the intake of water into the cell. How far the presence of a semi-permeable membrane is necessary for an understanding of water intake into the cell has still to be made clear.

Whatever may be the case with the non-vacuolated cell, there appears to be good reason for supposing that the intake of water by the vacuolated cell can be largely related to osmotic phenomena. Whether or not the outer layers of the protoplast are differentiated from the bulk of the protoplast so as to constitute semi-permeable membranes differing in permeability from the rest of the protoplast, it is reasonable to suppose that the essentially colloidal protoplast must itself act as a semi-permeable membrane between a liquid external to the cell and that contained in the vacuole.

4. EVIDENCE FROM THE FACTS OF SELECTIVE PERMEABILITY AND ALLIED PHENOMENA

A great number of observations have been made which indicate that different substances penetrate into cells at very different rates and to very different extents. This behaviour of the cell is directly comparable to the behaviour of the membranes considered in Chapter V,

and so it is very natural to account for this differential intake of substances into the cell by the presence of a superficial layer of the protoplasm which acts in the same way as an artificially prepared precipitation membrane. Other phenomena, such as the presence in the protoplasm of dissolved substances, including free electrolytes (Höber, 1912 *b*, 1913), which do not diffuse out into the external medium, and the deplasmolysis of plasmolysed cells in glycerol and various salt solutions, are also readily explained on the same assumption. Also artificial membranes of collodion impregnated with calcium phosphate (Meigs, 1913) or an ethereal extract of muscle (Philippson, 1913) have been prepared which behave very similarly to certain cells in regard to the passage of salts.

We have already seen when considering the evidence for the presence of a plasma-membrane yielded by the facts of the water relations of the cell, that there are those who deny the existence of such semi-permeable membranes. In the present connection reference may be made more particularly to the opinions of Moore and Roaf (1908), who state that the membrane theory fails to explain (*a*) the difference in composition of electrolytes in the corpuscles of blood and in the serum surrounding them; for instance, the concentration of chloride in the serum was found to be about three times the concentration of this ion in the corpuscles (and see also Abderhalden, 1898 and Höber, 1912 *a*, for further data); (*b*) the physiological effects of perfusion by media defective or excessive in certain electrolytes and normally present in the cell; and (*c*) the selective uptake of certain ions. Moore and Roaf hold that these effects are simply explained on the view that the cell proteins or other substances of the protoplasm form chemical or adsorption compounds with ions. The cell, when functioning normally, is held to be in a state of mobile equilibrium so that it can undergo reversible changes involving these electrolytic constituents, and presumably others, whereby they become associated with the cell protoplasm or the reverse. These writers thus suggest that selective uptake is to be explained, not by the selective permeability of a membrane, but by specific affinities of the cell protoplasm for certain ions, whereby they are adsorbed or combine chemically with the protoplasm, while the quantitative relation between the concentration of an electrolyte or ion in the cell and in the environment is maintained at a definite equilibrium which depends on the adsorption equation or molecular affinities, and which will therefore not as a rule correspond to equality of concentration inside and out. Similarly it is pointed out by Moore,

Roaf and Webster (1912) that if a cell is washed with a solution either free from, or very poor in, a particular ion, the amount of this ion washed out from the cell may be practically inappreciable, for it will only be washed out until the concentration of the ion in the external solution bears to the concentration of the ion in the cell the relation required by the adsorption equation or molecular affinities, and under these circumstances the equilibrium concentration of the ion in the external solution may be very low indeed.

In supporting the membrane theory, Bayliss (1915) deals particularly with the view that the apparent impermeability of cells to some salts can be sufficiently accounted for by the presence of a membrane semi-permeable as regards colloids, but permeable to crystalloids. This view does not differ greatly from that of Moore and his collaborators already considered. To such a view Bayliss raises the following objections. Firstly, as, if a membrane is permeable to one ion of an electrolytically dissociated salt and not to the other, it is impossible for the salt or either ion to pass through the membrane (see Chapter V), if we have a salt of a protein which is electrolytically (but not hydrolytically) dissociated, and the protein ion is incapable of passing through the membrane, the other ion of the compound will also be incapable of doing so. But if there are two colloidal salts present, in one of which the kation is diffusible and in the other the anion, as there must presumably be if a neutral salt is taken into the cell and both ions so held, then both ions will diffuse readily out from the cell, so that in such a case a hypothesis not involving a membrane exhibiting complete or partial semi-permeability to such salts will not explain the observed facts. Secondly, Bayliss thinks there is no satisfactory evidence for the presence of compounds between proteins and neutral salts. Thirdly, glucose does not form a compound with proteins of the form required by the hypothesis, but exists free in the blood according to Asher (1912), and is apparently indiffusible into the corpuscles under normal conditions. Fourthly, the high osmotic pressure of certain cells cannot be given by substances of such high molecular weight as proteins, and seems only accountable by the presence of substances of comparatively low molecular weight in an osmotic cell. Finally, difficulties arise with regard to the distribution of different ions within and without the cell if a membrane more or less impermeable to crystalloids is dispensed with. It has been shown both by Bayliss (1911) and Donnan (1911) that it is possible to have different concentrations of a salt on the two sides of a membrane through which the salt can diffuse freely, but that in

this case the facts of distribution differ very considerably from those in the case of blood serum and corpuscles, for example. Thus, if, for instance, the sodium salt of a protein is separated from pure water , by a membrane of parchment paper, the diffusible sodium ions can only diffuse to a position in which their osmotic pressure is balanced by the electrostatic attraction of the ,non-diffusible oppositely charged protein ion. Now if the pure water of the external medium is replaced by a solution of a freely diffusible salt, as for example potassium chloride, the potassium ions and chlorine ions can both diffuse through the membrane, and some of the potassium ions can replace the sodium ions in relation to the negatively charged protein ions, and this process will proceed until the sodium and potassium ions are distributed throughout both solutions in the same relative proportions. But the absolute concentrations of the two kations will be different on the two sides of the membrane. Now in the case of rabbit blood (Abderhalden, 1898) the potassium bears a different ratio to sodium in the plasma from that which it bears in the corpuscles, so that the presence of a colloidal salt within a membrane permeable to crystalloids does not account for the distribution of ions in this particular case.

In reviewing the evidence given by a consideration of the relation of solutes to the cell in favour of, or against, the presence of a semi-permeable plasma-membrane, it is again necessary to distinguish the cases of the vacuolated and non-vacuolated cell. Failure to do this in the past has undoubtedly led to the drawing of conclusions improperly. It has already been indicated that the protoplasm of the vacuolated cell fulfils the conditions necessary for a membrane between the vacuole and the external medium, and one which may exhibit differential permeability to different substances. The retention of sugar and the red pigment by the cells in slices of root of red beet can be explained satisfactorily on these grounds. Similarly the deplasmolysis of vacuolated cells such as those of *Spirogyra* and of the staminal hairs and epidermis of *Tradescantia* when plasmolysed in glycerol or solutions of certain salts is to be explained by the gradual passage of the solute through the protoplasm into the vacuole. In order to obtain evidence in relation to the presence of a plasma-membrane we shall do well to devote special attention to data derived from a study of the relation of solutes to non-vacuolated cells. Although Pfeffer (1900) admitted that no absolutely convincing proof had been brought forward, he gave reasons for supposing that the limiting membranes have different diosmotic properties from the

rest of the protoplast (Pfeffer, 1890, 1900). Thus, when a cell is treated with very dilute hydrochloric acid, the plasmatic membrane assumes "a condition of rigor," but its original diosmotic properties are usually at first retained, and a dye, which is unable to penetrate the plasma-membrane will rapidly penetrate throughout the dead protoplast if it can find entrance through a tear or gap in the membrane. The fact, already referred to, that the vacuolar membrane may actually be separated by strong and sudden plasmolysis, is cited as a further argument. The reader must form his own judgement of the value of these lines of evidence derived from pathological conditions; to the present writer they are very unconvincing, and it is to be noted that Pfeffer himself did not press them.

With regard to non-vacuolate cells, then, it must be admitted that the evidence is insufficient to enable one to come to any conclusive decision as to the presence of a limiting protoplasmic membrane. But here again, as with the water relations of the cell, it is impossible to avoid the conclusion that substances may be able to pass into the protoplasm and may be held there simply on account of the colloidal properties of the protoplasm. The cases dealt with by Bayliss are much simpler than those presented by living cells. When the state of equilibrium between the very complex system which constitutes the latter, and the external medium, is disturbed by changes in the composition of the external medium, it is clear that there may be expected far-reaching changes in regard to molecular association in the protoplasm, changes which may involve adsorption of substances from the outer liquid, chemical combination, or breaking down of existing molecular associations in the protoplasm to bring about a new condition of equilibrium. While the possibility of a limiting semi-permeable plasma-membrane is not ruled out, the evidence at present available from a consideration of the relation of the cell to substances dissolved in the medium outside it, is by no means overwhelmingly in favour of the presence of such a membrane.

An observation made by Osterhout (1913 *d*) on the marine alga *Griffithsia* may be taken as an example of one line of evidence adduced in support of the different permeabilities of the two surfaces of the protoplasm. If cells of this alga are placed in a solution having the same relative composition as sea-water, but stronger, plasmolysis takes place, and on returning the cells to normal sea-water the protoplasts regain their original size. But if instead of sea-water a solution of ammonium chloride is used as plasmolysing solution,

contraction of the protoplast from the cell wall also takes place, but the vacuole contracts much more so that the width of the protoplast may increase greatly at the expense of the vacuole.

Osterhout offers two possible explanations of this observation. Firstly, the outer surface of the protoplasm may be more permeable to ammonium chloride than the inner, or secondly, the ammonium chloride may produce an alteration in the permeability which causes a contraction called "false plasmolysis[1]." If this false plasmolysis of the inner surface is greater than that of the outer, the observed effect would result.

This observation may, of course, be truly explained on the basis of semi-permeable membranes surrounding both the protoplasm and the vacuole, but it might also be explained by supposing that the ammonium chloride altered the swelling capacity of the protoplasm as a result of which it absorbed water from the vacuole with the observed result.

5. Evidence derived from a Consideration of the Electrical Conductivity of Living Cells and Tissues

Living cells and tissues have a remarkably high electrical resistance (Stewart, 1897; M'Clendon, 1910), which rapidly falls if the cells or tissues are treated with a reagent bringing about the death of the cells (Osterhout, 1912 b; Stiles and Jørgensen, 1914 a). This phenomenon is easily explained by supposing that in the living cell the plasma-membrane offers considerable resistance to the passage of ions across it, but that when the cell is killed the organisation of the plasma-membrane is altered so that it becomes readily permeable to ions. That a visible change does often take place in the surface layer of the cell at death has already been noted.

Other explanations of these phenomena are however possible. Thus if electrolytes in the cell were held in molecular association with the cell colloids there would exist a system of high electrical resistance, while death of the cell might involve a breaking down of these compounds and the setting free of electrolytes. However, Höber (1910, 1912 b, 1913) has shown by ingenious electrical methods that free electrolytes are present in the cells of red blood corpuscles.

Such a contraction has been noted by Osterhout (1908 b, 1913 c) when cells are immersed in dilute solutions of various salts or even in distilled water. This evidently has nothing to do with true plasmolysis.

SUMMARY OF THE EVIDENCE FOR THE EXISTENCE OF
LIMITING SEMI-PERMEABLE PLASMA-MEMBRANES

Physico-chemical considerations indicate that the surface layer of the protoplasm must differ physically and chemically from the rest of the latter, the actual composition of the limiting layer depending not only on the composition of the protoplasm, but also on that of the medium with which the protoplasm is in contact. Any change in such a medium will bring about changes in the limiting layer. Ultramicroscopic, as well as microscopic, observations, including those made with the aid of microdissection, show that in some cells there may be a comparatively wide border (the hyaloplasm or ectoplasm) differentiated from the internal protoplasm, but that there may also be a much more delicate limiting layer which is regarded as the true plasma-membrane. These lines of investigation do not give definite information on the question whether the permeability properties of the limiting layer differ considerably from those of the internal protoplasm, except that as the surface layer should be more concentrated and viscous, it should offer greater opposition to the passage of dissolved substances (compare Chapter IV).

The relations of the cell to water and dissolved substances are generally regarded as yielding evidence of the existence of a semi-permeable membrane bounding the protoplast, but an analysis of the evidence indicates that it would be premature to draw such a conclusion from existing data. The low electrical conductivity of tissues affords some evidence of the presence of a semi-permeable membrane. In this connection, unfortunately, experimental observations are very scanty, while with plants observations on non-vacuolate cells appear to be non-existent.

Conclusions appear to have been drawn incorrectly in the past owing to the distinction between vacuolate and non-vacuolate cells not having been drawn with sufficient definiteness. In the case of the vacuolate cell there appears to be every reason to believe that the protoplasm acts as a membrane, to some extent semi-permeable, separating the vacuole from the external medium.

COMPOSITION OF THE LIMITING LAYER

This is a question that has given rise to very considerable discussion.

We have noticed that dissolved substances which lower the surface tension of the solvent tend to accumulate in the surface layer. For this reason the various constituents of the protoplasm may be expected to be present in the plasma-membrane. Among substances

present in the cell are various fatty substances which lower the surface tension of water considerably. These fatty substances include the true fats which are glyceryl esters of higher fatty acids, the lipines (Leathes, 1910), which are complex compounds of fatty acids with nitrogen-containing groups, and phospholipines containing phosphorus in addition, of which the best known is lecithin. Although Quincke (*1879, 1888, 1894, 1902*) had earlier conceived the protoplast as surrounded with a film of oil, undoubtedly the popularity of the theory of a lipoid plasma-membrane dates from the researches of Overton (1895, 1896, 1897, 1899, 1900, 1901) who showed in a series of investigations with a number of different cells and a large number of substances, including aniline dyes, that those substances soluble in lipoids easily enter the cell, while those which are insoluble in lipoids do not readily penetrate living cells. Meyer's theory of narcosis (1899) rests on a similar foundation.

In this connection Overton laid particular stress on the importance of lecithin and also cholesterol, which is not a true lipoid substance at all, but a complex alcohol (Windaus and Stein, 1904 *a*, *b*). Czapek (1910 *a*, 1910 *b*, 1911 *b*, 1914) on the other hand, rather emphasised the importance of the simpler neutral fats. He came to his conclusions as the result of a series of experiments on the exosmosis of tannin from plant cells, mainly those of the sub-epidermal mesophyll of the leaf of *Echeveria*, brought about by treatment with various toxic substances. Czapek came to the conclusion that, with few exceptions, for exosmosis to occur the concentration of the dissolved substance must be great enough to lower the surface tension of the solution (against air) to 0·68, that of pure water being taken as unity. He further found that a strong emulsion of neutral fat has a surface tension against air of 0·68, and that this is a minimal value; however strong the emulsion the surface tension is not lowered below this. Czapek was thus led to think of the surface layer of the cell as a fatty emulsion, which might indeed contain other substances, but of which the principal constituents are neutral fats. On surrounding the cell with a solution of lower surface tension than 0·68, a breaking down of the organisation of the semi-permeable membrane is supposed to result with consequent exosmosis of the contents.

Some substances, however, were found to produce exosmosis when presented to the tissue in solutions of considerably higher surface tension than 0·68. To such substances Czapek attributes a specific toxic action, the substances in question apparently acting chemically on the limiting membrane.

In a series of observations on the exosmosis of electrolytes from plant tissue, principally potato tuber, Stiles and Jørgensen (1917 *a*) have shown that it is impossible to find for any substance among a number examined, a critical concentration below which exosmosis will not take place. Exosmosis takes place in all concentrations examined, slowly at first, then more rapidly, and as equilibrium is approached, more slowly again. These stages are passed through more rapidly the more concentrated the solution of toxic substance. Czapek simply selected an arbitrary time and decided that if exosmosis had not taken place in that time it was not going to, with the exception that in some cases this time was arbitrarily extended; in other cases rapid exosmosis was attributed to secondary injury. Such methods of enquiry are not very convincing, and Koltzoff (1912) criticised Czapek for neglecting the time factor in his experiments. Czapek replied to this (1914) that he dealt only with equilibrium conditions and a time factor does not therefore enter into the question. But unless the progress of an action is followed with time, it is not always possible to decide whether equilibrium conditions have been reached. In those experiments of Czapek in which exosmosis had not occurred after the lapse of an arbitrary time, it is at least conceivable that prolongation of the time of action might have revealed exosmosis.

An important difficulty in Czapek's theory pointed out by Koltzoff and Vernon (1913) arises from the fact that the surface tensions of the solutions used in his experiments were measured against air, while in the experiments themselves it is the surface tension of the solution against the outer layer of protoplasm which is in question, while by no known means can the surface tension between two immiscible liquids be determined from their surface tensions against air. Hence it is not at all likely that even in Czapek's experiments those concentrations of different substances just producing exosmosis had the same surface tensions under the experimental conditions in question. The experiments of Stiles and Jørgensen have further shown that solutions which produce equal exosmosis of electrolytes have not necessarily the same surface tension against air. Having regard to this fact and to the exceptions to the rule recorded by Czapek himself, it seems clear that the permeability changes in the cell produced by toxic substances cannot be referred to surface tension alone.

Further arguments against the acceptance of Czapek's conclusions are that there appears no reason why a solution the surface tension

of which against air is less than that of protoplasm against air should bring about cytolysis, while finally Czapek himself quotes examples of fats, aqueous emulsions of which lower surface tension considerably below the critical value of 0·68.

An argument frequently advanced against the theory of a lipoid limiting membrane is that water, which enters most cells so readily, could not so pass through a continuous film of fatty substance. This argument, however, can scarcely be maintained against Czapek's view that the fat does not form a continuous layer, but is in the form of an emulsion. Also lecithin, on which Overton laid stress, when shaken up with water, forms an emulsoid system consisting of a less aqueous disperse phase in a more aqueous dispersion medium, and it is presumably in this form that the lecithin would be held in the surface layer of the protoplasm. Unfortunately for Overton's theory, lecithin in this form is not a solvent for lipoid soluble substances only (Nathansohn, 1904 a). Membranes of lecithin, and also of cholesterol, were found to be completely impermeable to all dyes examined, while when the lecithin membrane was saturated with water, dyes both soluble and insoluble in lipoids penetrated the membrane, not merely the lipoid-soluble ones (Ruhland, 1908 b). For a further discussion of the properties of lecithin and cholesterol in regard to cell permeability reference may be made to Bayliss (1915). Ruhland (1912 a, b, 1913 a, 1913 b, c, 1914) moreover, now holds that the intake or non-intake of dyes and other substances by plant cells depends rather on the size of the molecules or molecular aggregates of the dyes, and not on their solubility or otherwise in lipoid substances.

Pfeffer (1900) regarded the plasma-membrane as largely composed of protein, as indicated by the "fixation" of the limiting layer on treatment of the cell with various reagents such as dilute acids, mercuric chloride and iodine, and by microchemical tests applied to the membrane after fixation. The action of dilute salt solutions in rendering the cell more and more permeable (Pfeffer, 1877, 1890; de Vries, 1885) could also be explained as due to coagulation of protein in the membrane. Pfeffer realised that other substances might also be present in the limiting layer.

Robertson (1908) pointed out that Overton's theory is invalid for a variety of reasons, the most cogent of which is the fact that the relation between solubility in lipoid substances and power to enter the cell does not hold generally. Robertson found no correspondence between penetrability and power to enter cells in the dyes examined

by him. Robertson is of opinion that the maintenance of the integrity of the cell surface can be adequately accounted for by supposing that the cell is surrounded by a thin concentrated film of protein. Such films are thought to exist very generally, and perhaps universally, at the bounding surfaces of droplets of protein solutions (cf. also Ramsden, 1903). Robertson thinks it possible that a discontinuous layer of lipoid substance may underlie the outer film of protein.

Lepeschkin (1910 a, 1910 b, 1911 a, 1911 b), while agreeing that lipoid substances may be present in the plasma-membrane, adduces experimental evidence in favour of the importance of proteins therein. The protoplasm of plant cells coagulates at definite temperatures, the protoplasm of *Spirogyra* sp. at about 50·5° C., that of the epidermal cells of *Tradescantia* sp. at about 70·7° C. This behaviour is similar to that of proteins (cf., for example, Schryver, 1913). The coagulation is visible in plasmolysed cells under the microscope owing to the change from a shining homogeneous to a granular appearance. At the same time there is a sudden increase in permeability made obvious by the sudden shrinkage of the protoplast. Coagulation of the protoplast by organic substances can also be brought about in the same way as that of non-living proteins such as egg-albumin.

Lepeschkin consequently comes to the conclusion that the plasma-membrane contains both protein and lipoid substances in important quantities. He dismisses, however, the view of Nathansohn (1904 a) that the membrane consists of a mosaic of lipoid substances and protein responsible respectively for the intake of lipoid-soluble and water-soluble substances, since the entry of these two sorts of substances is not independent one of the other. Thus, the entry into the cell of salts and dyes soluble in water is diminished in the presence of narcotics soluble in fats. The value of this argument is, however, doubtful.

Lepeschkin's own view is that the plasma-membrane is largely composed of lipoid substances loosely combined with protein.

It will be observed that both Czapek and Lepeschkin worked with vacuolate cells, and there is no evidence to decide whether they are dealing with the whole thickness of the protoplasm, or only a thin limiting layer of it. Lepeschkin recognised this quite clearly, simply using the term plasma-membrane to designate that part of the protoplasm which is the seat of the phenomena he investigated. This plasma-membrane is the surface layer and an unknown thickness of cytoplasm within it, even possibly the whole thickness.

For an account in English of the work of Czapek and Lepeschkin,

brief, but containing all the essential facts, reference may be made to an article by F. F. Blackman (1912).

Osterhout (1911, 1913 *b*) concluded that the plasma-membrane, could not be lipoid in character because, contrary to the finding of Overton, Osterhout had shown that a large number of inorganic salts penetrate into the cells of *Spirogyra*. Further, although both sodium chloride and calcium chloride when presented in pure solution can enter *Spirogyra* cells, neither of these salts can penetrate into these same cells when present together in a solution in certain proportions. To this phenomenon of "antagonism" we shall return later. It will be sufficient here to remark that Osterhout regards his experiments as indicating a protein rather than a lipoid plasma-membrane.

In summing up the data here presented dealing with the composition of the limiting membrane of the protoplasm, it is clear that any view which regards the surface layer as composed exclusively of one kind of substance, either fatty or protein, must be dismissed at once. Both on theoretical grounds, and as the result of experimental work, it seems clear that the limiting layer of the cell must contain those constituents of the protoplasm and of the external liquid which lower the surface tension. These substances will thus constitute a colloidal complex, either a sol or gel, but how the molecules of the various constituents are arranged in reference to one another our present knowledge is insufficient to indicate.

Finally two points appear to deserve special emphasis. Firstly, on the view of the plasma-membrane here adopted, it is clear that the limiting membrane of the cell is not an invariable structure, but will vary with alterations in the composition both of the external medium and the protoplasm. It is clear that the hypothesis of de Vries, that the plasma-membranes are permanent structures never arising *de novo* but only from pre-existing membranes, is completely untenable.

Secondly, we can understand how it is impossible to separate the limiting membrane from the rest of the protoplasm. It depends for its existence on, and is closely related to, the media which it separates, and we should expect it to pass over into the two phases on either side of it, gradually in some cases, more or less sharply in others, according to the composition of the protoplasm and the external medium.

THICKNESS OF THE PLASMA-MEMBRANE

Seifriz (1921) has recently discussed this question. It is obvious that the plasma-membrane must be very thin, and that any measure-

ments must be very crude approximations. With this provision Seifriz concludes that the thickness of the plasma-membrane is of the same order of magnitude as that of precipitation membranes, namely $0 \cdot 1\mu$ ($0 \cdot 0001$ mm.).

OTHER CELL MEMBRANES

Some of the arguments for the presence of plasma-membranes bounding the vacuole and the outside of the protoplasm hold equally for the presence of such membranes between the protoplasm and nucleus, and the protoplast and chromatophores or plastids. Kite (1913 *a*) indeed, who regards plasmatic membranes as hypothetical structures, regards the nuclear membrane as definitely present. He describes it as a stiff gel and not to be confused with hypothetical structures such as plasmatic membranes. Accepting for the moment the membrane theory of cell permeability, an experiment described by Osterhout (1913 *d*) illustrates very prettily how such membranes may differ in permeability from the plasma-membrane. Cells of the marine alga *Griffithsia* contain chromatophores which are pigmented with chlorophyll and a red pigment soluble in water. Under normal conditions the pigment remains in the chromatophores, it being assumed the chromatophore membrane is impermeable to it. If the cell is treated with certain concentrations of ammonium chloride, the vacuole contracts while the outer surface of the protoplasm "still retains its full turgidity." At a certain stage in this process the red pigment diffuses out from the chromatophores but remains within the protoplasm, which thus becomes stained deep red. This is held to show the differential permeability of the chromatophore-membrane and of the plasma-membranes to the red pigment. It must not be forgotten that it would be possible to explain the phenomenon on the basis of changes in colloidal complexes without the invocation of membranes with differential permeability.

CHAPTER IX

THE WATER RELATIONS OF THE PLANT
CELL

I NTIMATELY connected with problems of cell permeability are the
water relations of the cell. Already in the preceding chapter in
considering the evidence for the existence of plasma-membranes it
has been necessary to touch on this subject. It has there been indi-
cated that the evidence for the existence of a semi-permeable mem-
brane forming the limiting layer of the protoplasm is very conflicting.
Hence in the case of the non-vacuolated cell it is not at all clear
whether it is correct to think of the cell with regard to its relations
to water simply as an osmotic cell, that is, as a solution of osmotically
active substances enclosed in a semi-permeable membrane, or whether
we are to compare it rather with a colloidal system such as a gelatin
or agar-agar gel or blood fibrin. It is true that M. H. Fischer (1908,
1910, 1915) has shown that certain animal tissues (frog's muscle and
sheep's eyes) behave similarly to gelatin and blood fibrin with regard
to the absorption of water, while MacDougal (1916 *a*, 1916 *b*, 1917,
1918, 1920, 1921) and MacDougal and Spoehr (1917 *a*, 1917 *b*, 1917 *c*,
1919, 1920) have succeeded in preparing colloidal mixtures of agar-
agar with gelatin, albumin or other proteins, urea or amino-acids,
in which agar-agar forms at least three quarters of the whole, the
so-called bio-colloids, which are very similar to plants in their be-
haviour towards water. Lloyd's work indicating a similar conclusion
has been referred to in the last chapter. But while we may be sure
that living plant protoplasm will imbibe water in the same way as

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a number of gels and other colloids, the evidence that this will com-
pletely account for the water relations of the non-vacuolated cell
lacks definiteness. A considerable amount of quantitative work is
necessary both on non-vacuolated plant cells and on colloidal systems
before the question can be regarded as in any way settled.

Similarly in the vacuolated cell, where the evidence that we have
to do with a system constituting an osmotic cell is more conclusive,
it is not at all clear that it is correct to regard the limiting layers of
the protoplast as semi-permeable membranes separating the proto-
plasm on the one side from an external solution contained in the
cell wall, and on the inner side from the liquid in the vacuole. On
the other hand, in the present state of our knowledge it does seem
reasonable to suppose that the whole layer of protoplasm may act
as a membrane separating vacuole and cell wall, and to some extent
semi-permeable inasmuch as it may allow the ready passage of water,
while varying greatly in its permeability to different substances dis-
solved in the water, and even completely preventing the passage of
some.

TURGIDITY

Whether on account of the swelling of the colloidal constituents
of the cell, or because the cell behaves as a solution of crystalloids sur-
rounded by a semi-permeable membrane, or on account of both these
reasons, under most circumstances the cell will absorb or has absorbed
water to such an extent, that it exerts a pressure on the cell wall,
which being capable of extension, is stretched. But as the cell wall
is elastic, that is, tends to return to its original form on being stretched,
it exerts a pressure against its extension, and consequently against
the increase in volume of the cell. The cell is therefore only capable
of absorbing water until the inwardly directed pressure resulting
from the stretched condition of the cell wall is equal to the
pressure sending water into the cells. The rigidity of the cell
resulting from this state of affairs has been not inaptly compared
with that of an inflated bicycle tyre or football (*e.g.* by Livingston,
1903). It is spoken of as turgidity[1], while the cell is said to be
turgid.

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[1] The term "turgescence" is sometimes used to express the same condition
of the cell (*e.g.* de Vries, 1874, and many later writers). The term "turgor"
is sometimes used in the same sense (*e.g.* by Gager, 1916). This term is some-
times used to express a definite quantity, but it is perhaps best to limit it to
its qualitative sense.

Omitting from our consideration the complications introduced if the protoplasm is in fact separated from cell wall and vacuole by semi-permeable membranes, which, in the absence of definite evidence, we are at least partly justified in doing, it is clear from what has been said in preceding chapters that in a full consideration of the permeability of the vacuolated plant cell to water we have to take into account the water relations of three distinct phases: the cell wall, the protoplasm and the vacuole.

It is generally assumed that the cell wall is completely permeable both to water and substances dissolved in the water, while it appears to be accepted that the protoplasm is readily permeable to water, but is impermeable or permeable in varying degrees to dissolved substances. The only part the cell wall is supposed to play in the water relations of the cell is to act as an elastic covering as described above, while the protoplast acts as a semi-permeable membrane enclosing the solution of salts and other crystalloids in the vacuole. This view of the cell I would speak of as the simple osmotic view, for it regards the living cell in its water relations as a simple osmotic cell. From what has been said in earlier chapters of the constitution of the protoplasm and cell wall it is obviously a considerable simplification of the actual state of affairs, neglecting as it does absorption of water and salts by the cell wall and protoplasm, with consequent changes in properties of these essential parts of the system, together with all electrical effects such as negative osmosis. Nevertheless the simple osmotic view is the one generally taken in discussions on the water relations of the plant cell, the reason for this being no doubt partly a natural preference for the simple rather than for the complex, partly to the beautiful work of de Vries (1884 *a*, 1885) which did so much to render the view popular, but no doubt also because in many cases the assumptions involved do not introduce errors of any considerable magnitude. This will most probably be the case where the cell wall is thin and the volume of the protoplast very small in comparison with that of the vacuole.

The simplest case we can consider is that of the single isolated cell. This is to be regarded as a solution of crystalloids (the cell sap) surrounded by a semi-permeable membrane (the protoplast), itself surrounded by the elastic cell wall. In the discussion immediately following it is assumed that the protoplast is absolutely semi-

permeable, a state of affairs rarely realisable in practice. When such a cell is immersed in a solution it is assumed that the latter permeates the cell wall so that the concentration of the solution in the cell wall is the same as that in the external solution. If the cell wall were absent, or if it were indefinitely extensible but completely inelastic, water would pass across the semi-permeable membrane until the difference in osmotic concentration on the two sides of the semipermeable membrane is zero. With water or very dilute solutions forming the external medium, water will pass into the cell in some cases until the latter bursts owing to the attenuation of the protoplasm, as we have already noted in the preceding chapter.

But with the presence of the elastic cell wall another force comes into play. We have already noticed that as the cell swells with absorption of water, the stretched cell wall exerts an inwardly directed pressure increasing with increase in stretching of the wall, and opposing the passage of water into the cell resulting from the difference of osmotic concentration on the two sides of the protoplasmic membrane. In the case of the turgid cell equilibrium will therefore be reached when the force sending water into the cell, that is, the difference in osmotic pressure of the internal and external solutions, is equal to this inwardly directed pressure exerted by the stretched cell wall. Before equilibrium is reached the pressure forcing water into the cell, that is, the difference in osmotic pressure of the internal and external solutions, is only partly compensated by the internally directed pressure of the stretched cell wall, so that the net pressure sending water into the cell is the difference in osmotic pressure of internal and external solutions less the inwardly directed pressure of the stretched cell wall. This net pressure obviously gets less and less as the difference in osmotic pressure inside and outside the cell is less and the pressure exerted by the wall greater.

The terminology relating to these water relations of the turgid cell is unfortunately somewhat confused. It will be noted that we are concerned with the following quantities. There is firstly the osmotic pressure of the external solution. This may be expressed in terms of molecular solutions of sucrose or potassium nitrate, or in the corresponding atmospheric pressure. Secondly, there is the osmotic pressure of the liquid in the vacuole, the cell sap. For this quantity a number of terms have been employed such as osmotic pressure, concentration of the cell sap, potassium nitrate or saltpetre value, turgor, turgor pressure and others. The term used here will be osmotic pressure when it is the pressure sending water into the cell

to which reference is made; but as the osmotic pressure can only exist when the solution is separated from another or from pure water by a membrane, a term is useful to indicate the concentration of substances in the cell sap in terms of the osmotic pressure that would be produced when such a solution is separated from pure water by a semi-permeable membrane. For this quantity the term osmotic concentration may be employed. Ursprung and Blum (1916 a) prefer to denote this quantity by the term "osmotic value," which also appears free from all academic objections.

Thirdly, there is the inwardly directed pressure of the cell wall (wall pressure) which is equal and opposite to the hydrostatic pressure exerted against it by the liquid in the cell. This hydrostatic pressure has also been spoken of as turgor or turgor pressure, but it will be observed that it is not the same quantity as the osmotic pressure for which the term is sometimes used. Here the term turgor pressure will be used to designate the total hydrostatic pressure exerted by the cell liquid against the protoplast and cell wall.

Fourthly, there is the net pressure sending water into the cell. This, as we have seen, is equal to the difference between the osmotic pressure of the external solution and the osmotic pressure of the cell, less the turgor pressure. This quantity is called by Ursprung and Blum (1916 a, 1916 d, 1916 e) and other writers in German (e.g. Höfler, 1920), often with the assumption that the external liquid is water, the suction force ("Saugkraft"), a term that has been applied to the net pressure sending water into whole organs and tissues. As this quantity is a pressure rather than a force it will here be termed the suction pressure (Stiles, 1922), and it will be convenient to distinguish between the net suction pressure, whatever the external liquid, and the full suction pressure[1] when the external liquid is water.

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[1] Ursprung and Blum (1916 d) clearly recognise the dimensions of the suction pressure as being those of a pressure, but for reasons which do not appear very forceful prefer to retain the term "Saugkraft." They say, "Was die Terminologie betrifft, so soll die Bezeichnung 'Kraft' beibehalten werden, obschon es sich ja um $\frac{\text{Kraft}}{\text{Fläche}}$ handelt, also um eine Grösse die nicht in Kg sondern in Atm. gemessen wird. Man pflegt ja auch in der Physik von Zug- und Druckkräften zu reden." Nor has the term "water absorbing power" proposed by Thoday (1918 b) for this quantity much to recommend it, for the quantity is certainly not a power but a pressure, and the term suction force has the claim of priority and the advantage of brevity, and in the form of suction pressure appears to be free from all disadvantages. When the term "suction pressure" alone is used in the following, it indicates the full suction pressure.

If P is the osmotic pressure of the liquid in the vacuole, P_e the osmotic pressure of the external solution, T the inwardly directed pressure exerted by the cell wall, and if S is the net suction pressure, the relation between these quantities is given by

$$S = P - P_e - T,$$

or, if the external liquid is pure water, so that $P_e = 0$,

$$S = P - T.$$

This relation was fully realised by de Vries (1884 a) who definitely states that the water attracting force of turgid tissue is not the same as that of the cell sap contained in its cells, but is smaller by a quantity corresponding to the elastic tension force of the protoplast and the cell wall.

When a cell is in equilibrium with pure water the turgor pressure is at its highest possible value; replacing the water with an osmotically active solution, for example, that of a sugar or a salt, must result in the passage of water from the cell as the equilibrium is disturbed by the increase in the osmotic pressure of the solution outside the cell. Consequently the turgor pressure is less and the cell comes to occupy less volume. With progressive increase in the concentration of the external solution the protoplast will contract and the cell wall along with it until a point is reached in which the cell wall no longer contracts along with the protoplast, so that a space appears between cell wall and protoplasm. As it is assumed that the cell wall is completely permeable both to water and dissolved substances, this space is filled with the external solution so that the unextended cell wall no longer exerts any pressure on the protoplast, the cell is flaccid and the turgor pressure is zero. The suction pressure in such plasmolysed cells is simply proportional to the difference between the osmotic pressures of internal and external solutions, and when equilibrium is attained the osmotic pressure of the cell sap is the same as that of the internal solution.

It has already been noted that when the cell is in equilibrium with water the turgor pressure is at its highest possible value, and the suction pressure (at equilibrium) being zero, the turgor pressure is equal to the osmotic pressure of the cell. At the beginning of plasmolysis the turgor pressure has reached its minimum value, that is, zero. Between these limits, the turgor pressure possesses intermediate values depending on the quantity of water taken into the cell. It has generally been assumed that within the limits of elasticity of the cell wall the tension of the wall increases proportionately

to the volume of water taken into the cell (Nägeli and Schwendener, 1877; Pfeffer, 1873; Thoday, 1918 b). Ursprung and Blum (1916 d) have shown, however, that such a relation only holds approximately. They point out that Sacerdote (1898) has shown that for a hollow cylinder with thin walls the increase in volume δV of the original volume V is given by the equation

$$\frac{\delta V}{V} = -3a\left[(1-2\sigma)\,p' - (1-\sigma)\,\frac{R}{2e}\,(p-p')\right],$$

where R is the radius of the cylinder, e the thickness of the wall, p and p' the pressures on the inner and outer sides of the wall, V the volume and a and σ are constants.

When R/e is very large the first term on the right side of the equation may be neglected, and in this case the increase in volume will be proportional to the increase in pressure. But if the first term is not negligible in comparison with the second, the linear relation between wall pressure and volume is only approximate. Diagrammatic representations of this linear relation are given by Thoday (1918 b) and Höfler (1920).

Assuming this linear relation, if V_z is the volume of the cell when the turgor pressure is zero, but before plasmolysis has commenced (the state referred to by Ursprung and Blum as "Grenzplasmolyse"), and if the osmotic pressure is P_z, and if when the turgor pressure (or wall pressure) is T the volume of the cell is V and its osmotic pressure P, neglecting the volume of the protoplasm, and assuming constant temperature, the following simple relations hold:

$$P_z V_z = PV \qquad \ldots\ldots\text{(Cf. Chapter VI)},$$

$$\frac{V - V_z}{V} = aT,$$

where a is a constant. The ratio V/V_z Höfler denotes by the term "degree of turgor stretching[1]."

If the volume of the protoplasm of the cell is not negligible in comparison with the volume of the vacuole, these equations require modification. If p represents the fraction of the whole volume of the cell in the non-turgid but unplasmolysed condition occupied by the protoplasm, and if the latter undergoes no change in volume

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[1] Höfler points out that the relation between osmotic pressure of the cell and turgor pressure is incorrectly given by Pantanelli (1904) and Höber (1914 a) following him.

during the intake of water by the vacuole, the former of the two equations just given becomes

$$P_z V_z (1 - p) = P (V - pV_z),$$

or, if v is the degree of turgor stretching,

$$P = P_z \frac{1 - p}{v - p},$$

the form in which the relation is given by Höfler.

Should the volume of the protoplasm also change with increasing turgor of the cell, this equation has to be further modified. In the absence of information on this point, however, further elaboration of this question is scarcely profitable.

It is to be observed that the relations given above only hold within the limits of elasticity of the cell wall. It must be admitted that we know very little about the elasticity of the cell wall. Schwendener and Krabbe (1893) immersed cylinders of young pith cells in water with the result that they swelled so that the length increased 25 to 30 per cent. On plasmolysis they contracted to the same length as that of similar cylinders plasmolysed immediately after their isolation from the plant without any immersion in water before plasmolysis. According to Pfeffer (1873) the staminal filaments of Cynareæ can be stretched to double their normal length without the limits of elasticity being exceeded. On the other hand, some cells, including bast fibres, are incapable of elastic stretching exceeding 0·5 to 1·5 per cent. of their normal length (Pfeffer, 1903).

Although a considerable amount of work has been done on the question of the elasticity of the cell wall, yet owing to the complex systems which constitute plant tissues, such work is full of difficulties and the results obtained open to considerable criticism. A discussion of this is out of the question here, but it may be pointed out that after considerable stretching the cell wall may take some time to return to its original shape, recovery being gradual (Detlefsen, 1884, 1888), while the modulus of elasticity will vary with the water content of the cell wall (Pfeffer, 1903). It is clear that relations involving assumptions with regard to the elasticity of the cell wall should be accepted with caution.

ISOTONIC SOLUTIONS AND ISOTONIC COEFFICIENTS

A solution having the same osmotic concentration as the cell sap is said to be isotonic with it, a solution of greater concentration than the cell sap is described as hypertonic, and one of lower concentration than the cell sap as hypotonic.

In the case of a cell without a wall in which it is assumed that the resistance to stretching offered by the protoplast is negligible, a cell immersed in a solution isotonic with the cell sap will undergo no change in volume, while in a hypertonic solution contraction, and in a hypotonic solution increase in volume of the cell will take place until the osmotic pressure of the liquid on the two sides of the protoplasmic membrane is the same.

In the case of turgid cells we have already noted that the suction pressure is less than the difference in the osmotic pressures by the turgor pressure, so that when, after immersion in a solution, no change in volume occurs in a turgid cell, the external solution is hypotonic, the tendency for water to enter the cell on account of the difference in osmotic pressure, being just balanced by the turgor pressure tending to force water out.

If now, a turgid cell is immersed in a solution of such a strength that a contraction of the protoplast away from the cell wall is just, but only just, brought about, the osmotic pressure of the solution external to the cell will be equal to that of the cell sap at the moment when the turgidity of the cell just disappears. It will be observed that the osmotic pressure will be somewhat greater than that of the turgid cell, as the latter has a somewhat greater volume than the cell at the commencement of plasmolysis, and consequently, as the total amount of solute in the cell remains unaltered, the osmotic pressure of the liquid in the vacuole must increase as the vacuole contracts.

If the concentrations of a number of different substances which just bring about plasmolysis of similar cells are determined, these different solutions should have the same osmotic pressure, and are therefore described as isosmotic. De Vries (1884 a) regarded such solutions as having the same attraction for water, so that the attraction of a molecule of any one substance for water would be inversely proportional to the concentration of its isosmotic solution. By the term *isotonic coefficient* of a substance de Vries (1884 a, 1888 a, 1889 a) signified the magnitude of the attraction of a molecule of the body in dilute aqueous solution for water. As unit he took one-third the attraction of a molecule of potassium nitrate.

De Vries concluded that the isotonic coefficient in strongly diluted solutions is a constant for every compound, the constant depending in a certain way on the composition of the compound and being expressible approximately by a simple number. The isotonic coefficients found by de Vries are given in the following table.

TABLE XVIII

Approximate Isotonic Coefficients of de Vries

Substances	Isotonic coefficient
Organic metal-free compounds	2
Alkali salts with one metallic atom in molecule ...	3
,, ,, two ,. atoms ,, ...	4
,, ,, three ,, atoms ,, ...	5
Alkaline earth salts with one metallic atom in molecule	2
,, ,, ,, two ,, atoms ,,	4

The value of the isotonic coefficient depends, of course, on the osmotic pressure, which depends, as pointed out in Chapter VI, on the degree of ionisation of the solution, the high values of the isotonic coefficients of electrolytes as compared with organic compounds such as sugar being explained on this ground. Since, as pointed out by Livingston (1903), the coefficients are only true values within limits (cf. Chapter VI), and as more accurate methods are available for the determination of the degree of ionisation, we may agree with Livingston that "it will be well for physiology when the practical use of these coefficients dies out entirely." Nevertheless, the work of de Vries on this subject was nearly as important for both physiology and physical chemistry as that of Pfeffer on osmotic pressure, and diagrams of unplasmolysed and plasmolysed cells taken from de Vries are to be found in standard text-books of botany and physics (*e.g.* Poynting and Thomson, 1905). Moreover, the methods elaborated by de Vries (1884 *a*, 1884 *b*) for the determination of isotonic coefficients have found a wide use for physiological problems connected not only with the water relations of the cell, but also with the passage of dissolved substances into and out from the cell. Some description of these methods is therefore desirable. They are the plasmolytic methods (the comparative plasmolytic method and the plasmolytic transport method) and the method of tissue tension.

Comparative plasmolytic method. Certain experimental conditions are desirable for the successful working of this method. These are (1) that the smallest trace of plasmolysis should be easily recognisable, (2) that all the cells of the tissue used should just plasmolyse in solutions of the same salt of exactly the same concentration, and (3) that the substances examined should not penetrate the cells. De Vries specially recommended three particular kinds of cells for this work, namely (1) the epidermal cells of the outer side of the growing leaf sheath of the dark red form of *Curcuma rubricaulis*, (2) the violet cells of the under epidermis at or near the midrib of

leaves of *Tradescantia discolor* (*Rhœo discolor*), and (3) red cells of the upper epidermis of the scales of the leaf stalk of *Begonia manicata*, together with cells of the red spots in the epidermis of the leaf ᛁ stalk in the neighbourhood of the scales. The last is not to be recommended so much as the other two but is particularly useful in the case of acids. The chief advantage of these particular cells is that they contain coloured sap in the vacuole which renders the observation of the slightest sign of plasmolysis much easier than in the case of cells with colourless sap.

It is a disadvantage of this method that individual cells are observed, and if different cells of the same tissue differ in their osmotic pressure observation has to be made on a number of cells. In order to reduce differences in osmotic pressure of different cells to a minimum, it is necessary to use cells as near together as possible. If the cells are not all of the same osmotic pressure, the solution in which half the cells become plasmolysed may be taken as the solution which is osmotically equivalent to the mean of the cell saps (cf. Darwin and Acton, 1901). A slight disadvantage is that the method requires time as the observer has to wait for equilibrium to result which is not likely to take less than an hour. An advantage of the method is that material for its performance can be obtained at all seasons of the year. The exactness of the determination depends on the degree of agreement between the different cells employed, on the number examined to determine the mean osmotic pressure of the cells and on the differences in concentration between the solutions of any of the substances examined.

Plasmolytic transport method. In this variant of the plasmolytic method cells are weakly plasmolysed in a solution of the salt under investigation and when plasmolysis is ended the cells are transferred to potassium nitrate solutions of different concentrations. That solution in which the volume of the plasmolysed protoplast remains unchanged is isotonic with the solution first used. This method has the advantage as compared with the comparative plasmolytic method that the same cell is used for the comparison of the experimental solution with the standard ones. It has the disadvantage that the long immersion of the cells in salt solutions may result in damage to the cells. De Vries regarded the method as useful chiefly in the investigation of the influence of concentration on isotonic co-efficients, as the method allows the use of a range of concentrations.

Method of tissue tension. For the practice of this method a piece of the growing stem or other elongated organ is cut out from a plant

and split longitudinally into four parts. Usually the pith elongates and the epidermis contracts owing to tensions in the tissue; consequently the strips of tissue curl with the pith occupying the convex side of the strip. If put into water the pith takes up water rapidly so that the whole rolls up into a spiral. If put in a strong enough salt solution the pith gives up part of its water so that the pith contracts and the curvature is reduced or may even be reversed, that is, the pith occupies the concave side. A solution of any particular substance can thus be obtained by sufficiently exact grading of the solutions in which no change of curvature takes place at all. In this concentration in which water is not taken up, the osmotic pressure of the external solution is equal to the osmotic pressure of the cells less the turgor pressure of the cells. Since these two last quantities may be assumed the same in similar strips of the same tissue, it follows that the solutions of different substances which just bring about no intake or excretion of water from the pith are isotonic with one another, though not with the cell sap. This method has two advantages. As a great number of cells are involved the values obtained are mean values. Also the experiment only takes a few minutes; indeed a long duration of the experiment must be avoided in order to prevent absorption of the salt or other dissolved substance by the tissue. According to de Vries it is a disadvantage of this method that the material for it can only be obtained in spring and summer, but there should be little difficulty in obviating this disadvantage where a warmed greenhouse is available. Details of the method in which scapes of dandelion (*Taraxacum dens-leonis*) are used as the experimental material in summer and hypocotyls of *Ricinus* seedlings are used in winter, are given in Darwin and Acton's *Practical Physiology of Plants* which is probably easily accessible to nearly all English readers[1].

[1] Darwin and Acton and other writers assume that the solution which produces neither increase nor decrease in curvature equals the cell sap in osmotic "force" (*i.e.* pressure), but as we have already seen, and as de Vries pointed out in his own description of the method, the osmotic pressure of the external solution which produces no change in the tissue is not equal to the osmotic pressure of the cell, but to the osmotic pressure of the cell less the turgor pressure. In the tissues used for the tissue tension method it does not follow that the turgor pressure is small enough to be neglected. This is immaterial in the determination of isotonic coefficients, for the osmotic pressures of the solutions of the different substances compared are in all cases presumed equal to the same osmotic pressure of cell sap less the same turgor pressure, and are therefore equal to one another. But the method does not give the osmotic strength of the cell sap, at any rate exactly. Thoday (1918 *b*) has accused Stiles and Jørgensen (1917 *b*) of falling into the same error when they speak of a solution in which disks of potato neither lose nor gain weight as

In Table XIX are given the exact values for the isotonic co-efficients of a number of substances found by de Vries by the plasmo-lytic and tissue tension methods.

TABLE XIX

Isotonic Coefficients of a Number of Substances
(Data from de Vries)

Substance	Isotonic coefficient found by	
	Plasmolytic method	Tissue tension method
Sucrose	1·88	1·84
Malic acid	1·98	---
Tartaric acid	2·02	---
Citric acid	2·02	---
Sodium nitrate	3·0	---
Potassium chloride	3·0	2·84
Sodium chloride	---	3·05
Ammonium chloride	3·0	---
Potassium acetate	3·0	---
,, dihydrogen citrate	3·05	---
,, oxalate	---	3·93
,, sulphate	3·9	3·92
,, monohydrogen phosphate	---	3·96
,, tartrate	---	3·99
,, malate	---	4·11
,, monohydrogen citrate	4·08	---
,, citrate	5·01	4·74
Magnesium malate	1·88	1·63
,, sulphate	1·96	1·78
,, citrate	3·88	3·53
,, chloride	4·33	---
Calcium chloride	4·33	---

DETERMINATION OF THE OSMOTIC PRESSURE OF THE CELL

Accepting the assumptions already made with regard to the water relations of the cell, the determination of the osmotic pressure of the cell sap is a simple matter. The method usually employed is the plasmolytic method of de Vries already described; in a second method, recently proposed and used by Höfler (1917), the osmotic pressure is also measured in the cell as such; other methods in which the osmotic pressure of the expressed sap is measured either directly

"approximately isotonic with the cell sap." But in such tissue as the potato tuber employed the turgor pressure is low in comparison with the osmotic pressure of the cell sap, and the solution in which such tissue neither gains nor loses weight is actually approximately isotonic with the cell sap (cf. Stiles and Jørgensen, 1919). With Thoday's "rejoinder" to this (1919), that it is incorrect to call a turgorless tissue turgescent, the joint authors would doubt-less agree, at the same time pointing out that it is not they, but Thoday, who has suggested that the potatoes used had a high turgor pressure, the word turgescent not even appearing in any of their papers.

or by one of the indirect methods indicated in Chapter VI have also been much employed for determining the osmotic pressures of plant cells, as, for example, by Dixon and Atkins (1910-1916), Harris and co-workers (1916-1921) and Mason (1919). These various methods will now be briefly described.

Plasmolytic method. This method is that of de Vries for determining isotonic coefficients and has already been described. Solutions of sucrose and potassium nitrate are the media generally used to produce plasmolysis of the cells investigated. The solution which just brings about plasmolysis, or that which just fails to bring it about, is regarded as isotonic with the cell sap, and accordingly has the same osmotic pressure. The values of osmotic pressure of solutions of sucrose over a wide range of concentrations have been obtained by Berkeley and Hartley and by Morse and his collaborators, and the existence of these determinations renders sucrose particularly useful as a plasmolysing substance in the plasmolytic method for the determination of the osmotic pressure of plant cells. A table of these values is given by Ursprung and Blum (1916 a). Sucrose is also to be recommended as it enters the majority of cells with extreme slowness and so does not materially affect the determinations on account of increased concentration of the cell sap resulting from endosmosis.

Immersion of the cells for some time is necessary for plasmolysis to be observed in many cases. Ursprung and Blum (*l.c.*) left herbaceous parts of a number of plants immersed in the solutions for 25 to 40 minutes or longer. Sections of stem and root of *Fagus* required at least 40 minutes. This rather long immersion involves the possibility of concentration changes in the cell sap owing to exosmosis from the interior into the surrounding solution, so that an error may arise on this account. As plasmolysis and deplasmolysis may result in tearing and stretching of the protoplasm it is advisable to use fresh cells for every determination. Change in volume which the cell may undergo when the tissue examined is isolated from the plant may also influence the result and should certainly be looked for and taken account of, and the same remark holds with regard to change in the volume of the tissue taking place during its immersion in the solution. The value of the osmotic pressure obtained is that of the cell at the moment when plasmolysis commences. If the osmotic pressure of the external solution is then P_z, the volume of the tissue V_z and its original volume in the turgid condition V, its osmotic pressure in that condition is $P_z V_z / V$.

A great advantage of the plasmolytic method lies in the fact that it 'allows the investigation of the osmotic pressure of individual cells; when the mean value of all the cells of a piece of tissue is required the concentration isotonic with the mean cell sap may be reckoned as that in which half the cells examined show definite plasmolysis.

Plasmometric method. This method is due to Höfler (1917); it is essentially a variant of the plasmolytic method, but in some cases, at any rate, is easier in execution. The essentials of the method are as follows. If a non-turgid but unplasmolysed cell is plasmolysed in a decidedly hypertonic solution, its volume, when plasmolysis is complete, is reduced by a definite fraction of the original volume, let us say by $1/n$. Assuming complete semi-permeability as in the case of the plasmolytic method, the concentration of the cell sap must have increased to $n/n - 1$ of its original value. Then if V_p is the volume of the plasmolysed protoplast and vacuole, V_z the original internal volume of the cell, P_e the osmotic pressure of the plasmolysing solution, the osmotic pressure of the cell is given by

$$P_z = P_e \frac{V_p}{V_z} \qquad \ldots\ldots(1),$$

and similarly if the cell was originally in a turgid condition with a volume V, the osmotic pressure is given by

$$P = P_e \frac{V_p}{V} \qquad \ldots\ldots(2).$$

The ratio V/V_z is the degree of turgor stretching (cf. p. 175) and so the last equation can be written

$$P = \frac{P_z}{G} \qquad \ldots\ldots(3),$$

where G is the degree of turgor stretching.

If it is assumed that the protoplast does not take part in the plasmolytic contraction the first and third of these equations become modified respectively to

$$P_z = P_e \cdot \frac{\dfrac{V_p}{V_z} - p}{1 - p} \qquad \ldots\ldots(4),$$

$$P = P_z \cdot \frac{1 - p}{G - p} \qquad \ldots\ldots(5),$$

where p is the proportion of the whole volume of the unplasmolysed but non-turgid cell occupied by the protoplast. Should the proto-

plast change its volume by a fraction αp the first equation becomes

$$P_z = P_e \cdot \frac{\dfrac{V_p}{V_z} - \alpha p}{1 - \alpha p}.$$

The quantity V_p/V_z is called by Höfler the degree of plasmolysis. In cells of regular geometric form in which there is a regular contraction on plasmolysis, both V (and V_z) and V_p are easily measured. In measuring the original volume of the cell it is necessary to measure the cell in a medium in which the cell undergoes no change in volume. For this purpose water and air are both ruled out, for in the former swelling may occur owing to intake of water, and in the latter evaporation of water may take place resulting in a diminution of volume. In air, moreover, the actual measurement is not easy. Ursprung and Blum (1916 d) recommend the use of liquid paraffin ("paraffin oil") for this purpose, as this substance neither reacts with nor enters the cell (Heller, 1904; Schilling, 1915). The cover glass must always be supported so that its weight does not fall on the cells or tissue and induce changes in form. The replacement of the paraffin by the plasmolysing liquid may be effected by irrigating the material on the slide, or by removing the material from the slide and washing in water. The former involves several successive washings to rid the cells or tissue of the paraffin, while the latter requires care to avoid deformation of the cells. Sucrose is to be recommended as the most generally useful plasmolysing agent for the reasons already given.

In a series of experiments in which parenchymatous cells from the stem of *Tradescantia elongata* were used, Höfler showed that the degree of plasmolysis was inversely proportional to the concentration of the plasmolysing solution, in the case of sucrose solutions varying in concentration from 0·30 to 0·60 gram-molecules a litre. With a number of solutions of different concentrations consistent values for the osmotic pressure of the cells were obtained, thus providing grounds for confidence in the correctness of the method.

Methods involving the determination of the osmotic pressure of the expressed sap. Many determinations of the osmotic pressure of the liquids in plant cells have been made by expressing the sap from the tissues and then determining the osmotic pressure of the sap by one of the methods mentioned in Chapter VI. The first point that calls for attention here is the method of extracting the sap from the tissues. Until comparatively recent the practice was to press the sap from the living untreated tissue (cf. Maquenne, 1896; Sutherst, 1901;

Cavara, 1901, 1905; Nicolosi-Roncati, 1909; Dixon and Atkins, 1910, 1912 *a*, 1912 *b*, 1912 *c*; Atkins, 1910; Marie and Gatin, 1912). A number of observations made by Dixon and Atkins among their earlier determinations led these authors to doubt the correctness of the assumption that the liquid so obtained represents an average sample of the sap. It had also been shown by André (1906 *a*, 1906 *b*, 1907 *a*, 1907 *b*) and by Marie and Gatin (1912) and confirmed by Dixon and Atkins (1913 *a*) that if a number of samples of sap are successively pressed from the same tissue, the samples exhibit a progressive increase in concentration and osmotic pressure. The explanation is that when pressure is first applied the protoplasm is not very permeable to the solutes in the vacuole, so that the first sample of liquid contains a much higher percentage of water than the vacuole. But the pressure results in the rupture of the cells and the passage out of the dissolved substances as well as the water. Consequently, as the pressure is increased to produce the successive samples of sap, more and more cells are burst so that the expressed sap becomes more and more concentrated.

In order to obtain an average sample of cell sap it is therefore necessary to treat the tissue so that the protoplasm is rendered completely permeable. Heat and toxic agents (toluene vapour and chloroform vapour) were tried but it was decided that the time required to render the cell membranes completely permeable was so long that enzyme or other actions might have sufficient play in the cells to alter materially the composition of the sap and so vitiate the results. Dixon and Atkins came to the conclusion that the most satisfactory way of rendering the cells completely permeable is to immerse the tissue in liquid air, as at this low temperature enzyme actions and autolytic and other changes are completely negligible. After such treatment the sap from successive pressings of the same piece of tissue had practically the same osmotic pressure. Subsequent experiments of Gortner, Lawrence and Harris (1916) have confirmed these conclusions.

For the determination of the osmotic pressure of the expressed liquid the method usually employed is that depending on the lowering of the freezing point of the solution. The thermo-electric method of determining the freezing point lowering evolved by Dixon (1911) may be mentioned in this connection. Harris and Gortner (1914) have published a useful table giving the values of the osmotic pressure for depressions of the freezing point between 0·001° and 2·999° C.

This method, of course, gives the mean osmotic pressure of all

the cells in a tissue or organ. This may be an advantage or disadvantage according to the end in view[1].

THE MAGNITUDE OF THE OSMOTIC PRESSURE IN PLANT CELLS

A great number of determinations of the osmotic pressures of plant cells and tissues have been made by the plasmolytic method and by determinations of the freezing point of expressed saps. With the vast array of data so obtained it is not proposed to deal in any detail here. Some reference should, however, be made to the magnitude of the osmotic pressure in plant cells, and to the variations in the osmotic pressure related to the ecological type of plant, the habitat of the plant, the position of the organ or tissue, the time of day and season of the year.

Unfortunately different authors have expressed the osmotic pressures of the cells examined in different units. Thus Sutherst simply gives the freezing point lowering, Ursprung and Blum give the concentration of sucrose or potassium nitrate isosmotic with the sap (the "cane sugar value" or "saltpetre value") while Dixon and Atkins and Harris and his collaborators give the pressures in atmospheres and Livingston (1903) in millimetres of mercury.

Pfeffer (1900), basing his remarks on older observations (de Vries, 1884 a; Stange, 1892; Janse, 1887 a), states that the osmotic pressure in the cells of land and fresh water plants is usually of the order of 5 to 11 atmospheres, but the recent observations of Dixon and Atkins, Harris and his collaborators, and Ursprung and Blum, clearly show that the normal range of osmotic pressures of plant cells is wider, extending much further in the upward direction. Thus out of 53

[1] The method of tissue tension has not been included among the methods for determining the osmotic pressures of cells for the reason explained in the footnote on p. 180; that is, on account of the presence of turgor pressure, any results obtained with turgid cells must necessarily be approximate, and with highly turgid tissues will be exceedingly incorrect. The same holds with regard to the change-in-weight (or change-in-volume) method. But for cells without an enveloping elastic envelope like the cell wall, a liquid in which cells neither lose nor gain in weight, that is, in which no interchange of water takes place, is to be regarded as isosmotic with the cell sap. The method is thus applicable to the determination of the osmotic pressure of some animal cells (H. J. Hamburger, 1886, 1887, 1889, 1890; Gryns, 1896; Koeppe, 1895, 1897; Löb, 1894; Hedin, 1895 a, 1895 b, 1896), and also to a few plant cells without cell walls.

By attaching weights to tissue, rendered turgorless by plasmolysis or death, until the tissue has regained its original length, it is possible, according to Pfeffer (1903), to calculate approximately in some cases the original osmotic pressure of the tissue. As such a method can only be very approximate at best, no further mention of it need be made here.

determinations tabulated by Dixon and Atkins (1913 *b*) the osmotic pressure was less than 11 atmospheres in only 13 cases, the lowest value found being 5·83 atmospheres in the leaf of *Saccharum offici-narum* and the highest 38·32 atmospheres in the fruit of *Vitis vini-fera*. According to Pfeffer (1900) the osmotic pressure, even in starved cells, rarely falls below 3·5 atmospheres (Stange, 1892; Copeland, 1896), while at the other end of the range saprophytic fungi, such as *Aspergillus niger* and *Penicillium glaucum*, growing on concentrated nutrient solutions, may develop an osmotic pressure of the sap of as much as 157 atmospheres (Eschenhagen, 1889). It is clear that in such cases the suction pressure is very great when the cells are transferred from the concentrated medium in which they were growing to distilled water, and it is not surprising that under such circumstances the cell walls may be ruptured (Pfeffer, 1900). The highest value recorded for the osmotic pressure of the sap of a flowering plant appears to be 153·1 atmospheres found by Harris, Gortner, Hofman and Valentine (1921) for a plant of *Atriplex con-fertifolia* growing in the neighbourhood of the Great Salt Lake.

THE RELATION BETWEEN THE POSITION OF A CELL IN THE PLANT AND THE OSMOTIC PRESSURE OF THE CELL

This is a question to which some considerable attention has been given, chiefly in relation to the ascent of water in trees. Ewart (1905) made some determinations of the osmotic pressure of leaf cells by the plasmolytic method and thought he had shown that the con-centration of the sap of leaf cells increases from lower to higher levels of the plant, but later (1906) came to the conclusion that the errors inherent in the method were too great to allow the drawing of definite conclusions. From the determinations of Dixon and Atkins (1910) it is concluded by Dixon (1914) that on the whole leaves at a lower level contain sap with a lower osmotic pressure than that of leaves inserted at a higher level, but that their results are not altogether satisfactory from this point of view. It is to be noted that these determinations were made before these workers realised the importance of a pre-liminary freezing of the tissues in order to obtain a real sample of the sap when pressed from the tissues. Hannig (1912), using the plasmolytic method, found the osmotic concentration higher in leaves than in roots. His statement that Dixon (1910) found the osmotic concentration of leaves independent of the level of their insertion is scarcely an exact statement of this author's results. Harris, Gortner and Lawrence (1917) made 26 sets of determinations, in-

volving material from 12 species of trees, of the osmotic pressures of
expressed saps from leaves inserted at different levels, and found
• almost without exception that the osmotic pressure is higher in leaf
cells the higher the insertion of the leaf. Some of their results are
shown in the following table. The slight exception recorded in the
table in the case of *Betula lutea* might easily be due to experimental
error.

<div align="center">TABLE XX</div>

<div align="center">Osmotic Pressure of the Sap of Leaves inserted at different
Levels. (Data from Harris, Gortner and Lawrence)</div>

Species	Height in feet	Osmotic pressure in atmospheres
Betula lutea	66	15·55
	52	16·01
	39	15·12
	25	14·11
	11	12·63
Quercus Prinus	47	20·23
	36	20·08
	30	19·72
	19	19·57
Robinia Pseudacacia	51	12·44
	39	11·07
	29	10·87
	9	10·68

An extensive series of observations made by Ursprung and Blum
(1916 *a*) by means of the plasmolytic method scarcely supports the
conclusions of Harris, Gortner and Lawrence, but the species used
were not the same; they were: *Helleborus fœtidus, Urtica dioica,
Fagus sylvatica, Sedum acre* and *Funaria hygrometrica.* Thus in the
case of *Fagus sylvatica* Ursprung and Blum could find no connexion
between the osmotic pressure of the cells and the height from the
ground of the leaves containing them, while generally the cells of
the same tissue (root, stem, leaf stalk and lamina) have a higher
osmotic pressure the nearer they are to the *base* of the organ. Other
conclusions of Ursprung and Blum are that cells of the same layer at
the same height from the ground have approximately the same os-
motic pressure if they are not too far apart, but neighbouring cells
belonging to different layers may differ considerably in their osmotic
pressures. In the cells of younger leaves the osmotic pressure was
less than in older leaves.

The highest values for the osmotic pressure were found in *Helle-
borus* and *Urtica* in the palisade. The same was the case with *Fagus*,
but here equally high values were found in the palisade, wood

parenchyma and medullary rays. Minimum values were found in the lower epidermis of the leaf in *Helleborus* and *Fagus* and in the cortex of the leaf stalk in *Urtica*. The mean values found by Ursprung and Blum for *Fagus sylvatica* are shown in the following table. The osmotic concentrations are given as concentrations of potassium nitrate.

TABLE XXI

Mean Values of the Osmotic Concentrations in the Cells of Different Tissues of *Fagus sylvatica*

(Data from Ursprung and Blum)

Organ	Epidermis	Spongy tissue	Palisade	Outer cortex	Inner cortex	Phloem paren-chyma	Com-panion cells	Cambium	Xylem parem-chyma	Cortical medul-lary rays	Xylem medul-lary rays
Leaf lamina	0·371	0·571	1·017	—	—	—	—	—	—	—	—
Branch	—	—	—	0·667	0·671	0·573	0·721	0·64	1·008	0·808	0·954
Stem	—	—	—	0·696	0·696	0·562	0·746	0·638	0·963	0·929	0·921
Root	—	—	—	0·65	0·671	0·583	0·70	0·625	0·979	—	—

DIFFERENCES OF OSMOTIC PRESSURE IN SPECIES
OF DIFFERENT HABIT

While the numbers quoted in the preceding section are sufficient to show that great variations of osmotic pressure may occur in different cells of the same species, it is certainly true that differences in the osmotic concentration of cells occur in relation to specific differences. Such differences are frequently related to the habit of the plants under consideration. Thus in succulents the osmotic pressures of the cells are relatively lower than in meso-phytes (Ursprung and Blum, 1916 *a*). In an investigation on the plants growing in the deserts of Arizona, Harris, Lawrence and Gortner (1916) found considerable variation of osmotic concentra-tion of different species, the different groups in order of increasing concentration being (1) winter annuals, (2) perennial shrubs, (3) dwarf shrubs and half shrubs, and (4) shrubs and trees. In a later investigation on the osmotic concentration of the leaf sap of plants from the Blue Mountains of Jamaica, Harris and Lawrence (1917 *b*) found that the osmotic concentration of the leaves of woody plants was greater than that of leaves of herbaceous plants, and the same result was obtained with plants of the Arizona deserts and the north shore of Long Island (Harris, Gortner and Lawrence, 1921 *a*, 1921 *b*). Ursprung and Blum (1916 *a*), however, found the osmotic concentration of the palisade cells of *Urtica dioica* about the same

(1·015 M KNO$_3$) as that of *Fagus sylvatica* (1·017 M KNO$_3$), while the mean values for epidermis and spongy parenchyma were higher ₊in both *Urtica dioica* and *Helleborus fœtidus* than in *Fagus sylvatica*.

That there might be some fundamental relation between the osmotic concentration of the cells of parasitic plants and their hosts was suggested by MacDougal and Cannon (1910) and MacDougal (1911 *a*, 1911 *b*). Since then observations by Senn (1913), and a number of observations by Harris and Lawrence (1916) and Harris and Valentine (1920) on montane rain-forest and desert Loranthaceæ indicate that the osmotic concentration of the cells of the parasite is in general higher than that of the leaves of the host. The values set out in Table XXII, taken from Harris and Valentine, indicate the degree of difference in the osmotic pressures of the cells of host and parasite.

TABLE XXII

Osmotic Pressures of the Cells of Host and Parasite
(Data from Harris and Valentine)

Parasite and host	Osmotic pressure of leaf sap of host in atmospheres	Osmotic pressure of sap of parasite in atmospheres
Phorodendron Californicum		
on *Acacia greggii*	26·57	33·66
on *Olneya tesota*	25·24	26·98
Phorodendron Cockerellii		
on *Populus wislizeni*	22·04	23·05
on *Salix wrightii*	20·88	24·98
on *Fraxinus attenuata*	23·47	26·47

Harris (1918) has shown that epiphytes have an abnormally low osmotic pressure in their cells, the osmotic pressure in the epiphytic species examined by him being 37 to 60 per cent. lower than that of the expressed sap of herbaceous forms, and 28 to 45 per cent. lower than that of ligneous forms.

Thus the evidence available appears to indicate that the osmotic pressure of plant fluids depends in part on the ecological type of plant. Harris and Popenhoe (1916) even claim to have shown a slight difference in the osmotic pressures, as indicated by freezing point lowering, of the expressed sap of the leaves of different horticultural types of the avocado (*Persea americana*).

THE INFLUENCE OF EXTERNAL CONDITIONS ON THE OSMOTIC
● PRESSURE OF PLANT CELLS

This question is naturally connected with that discussed in the ,
last section, for differences correlated with the ecological type of a
plant may possibly be traced back ultimately to differences in the
habitat. Thus Harris, Gortner, Hofman and Valentine (1921) found
abnormally high osmotic values for the expressed sap of plants
growing in the neighbourhood of the Great Salt Lake, the sap in two
plants of the salt desert half shrub *Atriplex confertifolia* growing on
rocky cliffs possessing osmótic pressures of 82·9 and 94·7 atmospheres
respectively while three plants of the same species growing on low
ridges in salt flats gave values of 74·2, 118·5 and 153·1 atmospheres
respectively. The great difference in the osmotic pressure of the sap
of plants of rain forest and of desert, even when the plants are of
similar growth forms, may be correlated with differences in the
environmental factors. Harris and Lawrence (1917 *b*) found the
average osmotic pressure of the sap of ligneous forms in the rain
forest of the Blue Mountains of Jamaica was about 11·44 atmospheres
as compared with 14·40 for ligneous plants growing in Long Island
and 24·97 atmospheres in the case of plants growing in the deserts
of Southern Arizona (Harris, Lawrence and Gortner, 1916). Similar
differences were found with herbaceous forms, the average osmotic
pressure of the sap of Blue Mountain, Long Island and Arizona
desert forms being 8·80, 10·41 and 15·15 atmospheres respectively.
High values of osmotic pressure in leaves of desert plants have also
been observed by Fitting (1911) and Keller (1913).

Iljin, Nazarova and Ostrovskaja (1916) measured the osmotic
pressure of cells of the roots and leaves of a number of swamp,
meadow and steppe plants by means of the plasmolytic method, and
compared the values obtained with the conditions of the surrounding
medium, soil or air, as the case might be, as regards water content.
In roots they found the highest osmotic pressures in steppe plants,
lower values in meadow plants, and lowest values in swamp plants,
the values obtained for these three groups of plants being respectively
0·40–0·48, 0·19–0·30 and 0·13–0·20 of the osmotic pressure of a
normal solution of sodium chloride. The water content of the soil or
swamp was in inverse order, so that it appears that the osmotic
pressure of root cells is less in roots growing in a soil with a higher
water content. Observations on a number of plants common to
steppe and meadow confirmed this. Thus in the cases of *Poa pra-
tensis, Triticum repens, Kœleria gracilis, Stipa capillata, Festuca*

ovina, and *Lythrum virgatum*, the osmotic pressure of the cells of the root was always higher in the plants of any one species growing in a drier soil than in roots of plants of the same species growing in a soil of higher water content. Similar relations between the water content of the soil and the osmotic pressure of the cells of root and shoot of maize have been found by McCool and Miller (1917) by the expressed sap method.

Iljin, Nazarova and Ostrovskaja found that the osmotic pressure of the cells of the root is no criterion of the osmotic pressure of the cells of the leaves. They come to a conclusion similar to that in regard to the influence of the water content of the soil on the osmotic pressure of the cells of the root, namely, that the water content of the air surrounding the leaves influences the osmotic pressure of the leaf cells: the higher the water content of the air the lower the osmotic pressure of the leaf cells. Thus leaves of the same plant, for example, *Senecio doria*, *Centaurea scabiosa* and *Sanguisorba officinalis*, had all the same osmotic pressure in their cells when growing in the open and therefore with all the leaves under the same external conditions as regards moisture, but when growing among grass the lower leaves always gave lower values of the osmotic pressure than the upper leaves. Drabble and Lake (1905, 1907) also found by the plasmolytic method higher osmotic pressures in the epidermal cells of leaves of those plants most subjected to factors tending to loss of water by transpiration.

Confirmatory evidence of the influence of water content of the soil on the osmotic pressure of the plant sap is forthcoming from the results of Pringsheim (1906), Meier (1915), Ursprung and Blum (1916 c) and Iljin, Nazarova and Ostrovskaja (1916). Plants of a number of species were grown by these various investigators under different conditions of humidity and the osmotic pressure of the sap of the various experimental plants examined. The result is quite general that higher water content of the environment corresponds with lower osmotic pressure of the cell sap, and *vice versa*. Even as long ago as 1884 de Vries recorded a higher osmotic pressure for shoot apices of *Helianthus tuberosus* after dry weather than after a long period of daily watering.

An increase in the concentration of substances dissolved in an aqueous medium is of course equivalent to a decrease in the water content. From the observations just recorded it is therefore not surprising to find that plants growing in strong solutions of osmotically active substances generally exhibit an increase in the osmotic pressures of their cell sap. Thus Janse (1887 a) showed that the osmotic

concentration of the cell sap of *Chætomorpha* increased as a result of transferring it to a solution of higher salinity, while Mayenburg (1901) found a similar increase in osmotic pressure in *Aspergillus niger* when this fungus was grown on strong nutrient solutions. The osmotic pressure of the cell sap of root hairs of halophytes appears to be considerably higher than that of mesophytic plants (cf. Drabble and Lake, 1905, 1907), while T. G. Hill (1908) has shown that the osmotic concentration varies with that of the external medium and in the same direction. It is, however, not at all clear whether the increase in osmotic concentration is due to simple absorption of the salt or other osmotically active substance from the external medium, or to a complicated series of internal changes not understood (cf. T. G. Hill, 1919, for halophytes; Mayenburg, 1901, for *Aspergillus*). According to von Faber (1913) the osmotic concentration of the cell sap of mangroves is considerably higher than the normal, but only in some cases is this due to a high concentration of sodium chloride. Stange (1892) could find no accumulation of potassium nitrate in the cells of the root tips of *Pisum* and *Lupinus* growing in strong solutions of this salt although the parenchyma cells behind the root tip contained the salt in considerable quantity.

Wind, no doubt by its influence on transpiration and consequent removal of water from the leaf cells, induces an increase of osmotic pressure in them (Ursprung and Blum, 1916 c).

The data bearing on the influence of temperature on the osmotic pressure of plant cells have been brought together by Ursprung and Blum (1916 c; see also Blum, 1917). From their own observations and the earlier ones of Copeland (1896), Pantanelli (1904), Lidforss (1907), Kny (1909), Winkler (1913), Buchheim (1915) and Meier (1915) it would appear that the osmotic pressure of the cells examined is a minimum in the region of 10° to 12° C. and increases with both increase and decrease in temperature. Yet the influence of temperature has not been wholly disentangled from the influence of other factors, and it would be premature to accept such an opinion as conclusively proved.

With regard to the influence of light on the osmotic pressure of plant cells, de Vries (1884 a) and Copeland (1896) have shown that etiolated plants give lower values of the osmotic pressure in their cells than normal plants. Buchheim (1915) concluded from observations on the alga *Cylindrocystis* that strong illumination brought about an increase in the osmotic pressure of the cell sap of this plant. Dixon (1914) found that illumination increased the osmotic pressure

in leaf cells and attributes this, no doubt correctly, to the formation
of carbohydrates. Meier (1915) found in a number of species a some-
what higher osmotic pressure in the leaves exposed to the sun than
in leaves of the same species growing in shady habitats. Observations
by Ursprung and Blum (1916 c) on *Funaria* showed the same relation,
while the same authors found higher osmotic pressures in epidermal
cells and most of the mesophyll cells of sun leaves of the beech as
compared with the corresponding values found in shade leaves of the
same species. Intense illumination thus appears to lead to an increase
in the osmotic pressure of leaf cells, as is indeed generally supposed.

PERIODICITY IN THE VALUES OF OSMOTIC PRESSURE IN PLANTS

The various factors of the environment undoubtedly influencing
the osmotic pressure of plant cells, it is only to be expected that in a
climate with a daily and annual periodicity there should be a corre-
sponding periodicity in the value of the osmotic pressure of the cell
liquids. A daily periodicity in the value of the osmotic pressure of
the cells of the different tissues and in different parts of the plant
has been shown by Ursprung and Blum (1916 b) in *Helleborus fœtidus,
Fagus sylvatica* and *Sedum acre* and also in the leaves of *Funaria,* the
osmotic pressure increasing from early morning until the afternoon
and then falling until early the next morning. This course runs
parallel with the temperature and inversely as the humidity.

An annual periodicity has also been made out by the same
authors, their results indicating on the whole minimum values in
the summer and maximum values in the winter. There are, however,
many departures from this, as would be expected when one considers
that the changes in the various environmental factors are by no
means regular.

THE SUBSTANCES IN THE PLANT RESPONSIBLE FOR THE
OSMOTIC PRESSURE

It has been noted in Chapter VI that an osmotic pressure can
only be developed when two solutions are separated by a membrane
which is impermeable, or only little permeable, to solute or solutes
on one side of the membrane. On the simple osmotic view of the
plant cell we have therefore to suppose that in the vacuole there are
present solutes to which the protoplasm is impermeable, and that
these solutes are in such concentration as to give rise to the high
osmotic pressures noted in previous sections of this chapter. To de-
termine the substances responsible for the osmotic pressure de Vries

(1884 *a*) analysed the expressed sap from a number of plant organs and calculated the osmotic pressure due to each of the substances present. He found the sum of the osmotic pressures so obtained approximately equal to the osmotic pressure of the expressed sap as determined by the plasmolytic method. A number of his results are summarised in Table XXIII.

TABLE XXIII

Osmotically active Constituents of Cell Sap

(Data from de Vries)

Osmotic pressures (as saltpetre values) of the various constituents in different species

Constituent	*Heracleum sphondylium* Leaf Stalk	*Gunnera scabra* Leaf stalk Young	*Gunnera scabra* Leaf stalk Old	*Rheum officinale* Stem	*Rheum hybridum* Leaf Stalk	*Rochea falcata* Leaf	*Rosa hybrida* Petal
Organic acids	0·020[1]	0·023[1]	0·028[1]	0·063[2]	0·124[2]	0·055[1]	0·023[3]
Potassium salts of organic acids	0·013	0·004	0·004	0·012	0·013	0·004	0·012
Glucose	0·152	0·026	0·021	0·085	0·052	0·030	0·218
Potassium chloride	—	0·062	0·090	—	—	—	—
Sodium chloride	0·014	—	—	—	—	0·015	—
Potassium phosphate	—	—	0·003	0·012	0·007	—	—
Total	0·199	0·115	0·146	0·172	0·196	0·104	0·253
Found saltpetre value of sap	0·22	0·12	0·16	0·20	0·22	0·13	0·27

[1] Principally malic acid and calculated as such.
[2] Principally oxalic acid and calculated as such.
[3] Not identified with certainty but probably malic acid and calculated as such.

These results and others show that the osmotically active substances of the cell sap are very varied. De Vries (1879, 1883) had previously emphasised the importance of organic acids in the maintenance of turgor, but his later work showed that this is by no means a general rule. Nevertheless, in *Rheum hybridum* oxalic acid, and in *Rochea falcata* malic acid, play a leading part in maintaining the osmotic pressure of the cell sap, while the observations of Kraus (1886) on a number of Crassulaceæ show that in these plants half the dry weight may consist of soluble salts of malic acid. High percentages of oxalic acid in the sap were found by de Vries in the cases of leaf stalks of *Begonia Rex* and *B. manicata,* in which free oxalic acid and potassium oxalate accounted together for about the osmotic pressure of the sap.

The percentage of the osmotic pressure of cell sap due to sugars was found by de Vries to be very varied. The sugar is always calculated as glucose by de Vries, but it will probably be more accurate to regard his numbers for glucose as approximately those for total sugar,

although of course where sucrose is present the osmotic pressures calculated on the assumption that the whole of the sugar is glucose are too high as the osmotic pressures of equal weights of sucrose and glucose are in the ratio of approximately 1 : 2. As free acids were present in the sap and as the method of analysis of de Vries involved heating the sap it is probable that any sucrose would be inverted and so appear as hexose reducing sugar. The highest values for glucose were found in the petals of *Rosa* and the leaf stalks of *Heracleum sphondylium*, in which the percentage of the osmotic pressure attributed to glucose was found to be 80·7 and 69·1 per cent. respectively. At the other extreme come leaves of potato in which the part of the osmotic pressure of the cell sap attributable to sugar was found to be less than 4 per cent. Between these two extremes all possible intermediate conditions can be found.

Potassium chloride is responsible for more than half the osmotic pressure in the cells of leaf stalks of *Gunnera scabra*, while in other plants other inorganic salts may form an important fraction of the total osmotically active substances, as for instance in growing shoot apices of *Helianthus tuberosus*, where de Vries found potassium nitrate responsible for 41·4 per cent. of the total osmotic pressure. Copeland (1896) concluded that the same salt was mainly responsible for the osmotic activity in *Fagopyrum, Pisum, Phaseolus* and *Zea*. The work of Nathansohn (1901) on *Codium tomentosum* suggests that in this plant sodium chloride may be an active osmotic constituent of the cell sap, while the more recent work of Wodehouse (1917) indicates the presence of a number of inorganic salts in the sap of the marine alga *Valonia*. It is probable that in marine plants generally, and also in halophytes, sodium chloride particularly may play a prominent part in the maintenance of osmotic pressure of the sap. That such is indeed the case cannot, however, be accepted without question (cf. T. G. Hill, 1919). Von Faber (1913) states that in mangroves the high osmotic pressures are due to sodium chloride in some species but not in others.

It will be noted that a dissolved substance in the cell sap can only be a factor in the maintenance of a permanent osmotic pressure if the protoplasm is for all practical purposes impermeable to the substance. Now it is to be expected from the general considerations in earlier chapters that substances of small molecular weight such as potassium chloride and potassium nitrate and other inorganic salts would penetrate the protoplasm with comparative ease and rapidity as they do through membranes artificially prepared. There

is undoubtedly a very real difficulty here to which it will be necessary to return later. It is an attractive theory to suppose that whereas the protoplasm is readily permeable to inorganic salts, it is impermeable to organic substances with larger molecules such as carbohydrates and organic acids, and that the osmotic pressure of the cell sap is to be attributed to these. This will, however, not explain the results of de Vries and Copeland recorded above.

Calculations of the molecular weight of the solutes in the expressed sap of seedling peas made by Maquenne (1896) by means of determinations of the freezing point, suggest an average molecular weight of 239, considerably higher than that of glucose, namely 180. On the other hand, the molecular weight of the solutes in the sap of *Helianthus* was found to be on the average only 136, and as glucose was present in the sap, it would appear that the osmotic pressure must be ascribed in large part to substances with lower molecular weights than that of glucose. Rather similar values were obtained by Dixon (1914) for the mean molecular weight of the solutes in the leaf sap of *Wistaria sinensis* and *Ulmus campestris*, the values in the former varying from 149 to 169, and in the latter from 148 to 165.

Comparison of the electrical conductivity of saps, which gives an approximate measure of the content of electrolytes, with the osmotic pressure, shows that there is no constant relation in different species between the content of electrolytes and total osmotically active solutes (Harris, Gortner and Lawrence, 1920, 1921 *a*, 1921 *b*).

THE SUCTION PRESSURE

Although the amount of water which a cell is capable of absorbing must depend on the osmotic concentration of the cell sap and the impermeability of the protoplasmic membrane, it is suction pressure which gives a measure of the pressure with which water is absorbed by the cell. Attention has recently been directed to the suction pressure in plant cells by Ursprung and Blum, who describe methods for determining the full suction pressure (1916 *d*) and who have published the values they have determined for it in a number of cases.

DETERMINATION OF THE FULL SUCTION PRESSURE

Ursprung and Blum (1916 *d*) describe two methods for the determination of the full suction pressure. These may be described as the indirect plasmolytic method and the equilibrium method.

Indirect plasmolytic method. It will be recalled that the full suction pressure equals the osmotic pressure of the cell sap less the wall

pressure. The indirect plasmolytic method consists in (1) measuring the osmotic pressure of the cell by plasmolysis as already described, and (2) in estimating the wall pressure. This is done by assuming that a linear relationship holds between wall pressure and cell volume between the extreme limits when the wall pressure is zero at plasmolysis and when the wall pressure is a maximum and equal to the osmotic pressure of the cell sap, when the cell is in equilibrium with pure water. It has already been pointed out that such an assumption can only be regarded as an approximation.

This method, it will be observed, involves the measurement of the original volume of the cell, its volume at plasmolysis, and its volume when fully swelled in distilled water. It is also necessary to determine the osmotic concentration of the cell.

Equilibrium method. The principle of this method was clearly enunciated by de Vries (1884 *a*) in reference to the determination of the suction pressure of transpiring shoots, but there are apparently no records of any measurements made by him. It consists simply in determining that concentration of a solution of a substance to which the protoplast is impermeable in which the cell undergoes no change in volume. In this case the net suction pressure is zero, so that if P_e is the osmotic pressure of the external solution, P the osmotic pressure of the cell sap and T the wall pressure,

$$0 = P - P_e - T,$$

or $$P_e = P - T \qquad \ldots\ldots(1).$$

When the cell is placed in distilled water, if S is the full suction pressure which is then exerted,

$$S = P - T \qquad \ldots\ldots(2),$$

so that, as T is the same in equations (1) and (2),

$$S = P_e.$$

Hence the full suction pressure of a cell is equal to the osmotic pressure of a solution of a non-penetrating substance in which the cell undergoes no change in volume. When the suction pressure of tissues is in question it will generally be found simpler to use weight as a criterion instead of volume.

Whichever method of determination is used solutions of sucrose are likely to be the most generally useful external media. It is also to be noted that the method neglects any external forces which may be operative such as tissue tensions. If there should be acting on a cell in the intact organ an external pressure of the same order of magnitude as the suction pressure found for the cell isolated from

the tissues when the external pressure is no longer acting, it is clear that the suction pressure so found gives no measure of the suction pressure of the cell in the uninjured organ. It is therefore necessary to confine the determinations to cells which are under no appreciable external pressure in the intact plant, or else to take account of such pressure.

THE MAGNITUDE AND RANGE OF SUCTION PRESSURES IN PLANTS

Ursprung and Blum (1916 *e*) have investigated the range of suction pressures in roots and shade leaves of *Fagus sylvatica*. Their chief results are as follows.

In all the leaves examined Ursprung and Blum find differences in the full suction pressures of the cells of different tissues, the order being under epidermis, upper epidermis, guard cells, spongy tissue, palisade. In all these tissues the suction pressure was found to increase with increasing distance from the ground. The values found for root apices 40 cms. below the level of the ground were the lowest of all, but root apices 10 cms. below ground gave abnormally high values. Apart from these last, which are completely unexplained, the mean values given by Ursprung and Blum's determinations are summarised in Table XXIV.

TABLE XXIV

Full Suction Pressures in Cells of *Fagus sylvatica*
(Data from Ursprung and Blum)

Distance from root apex in metres	Suction pressures in atmospheres					
	Root apex	Upper epidermis	Under epidermis	Guard cells	Spongy paren-chyma	Palisade
0·0	5·3	—	—	— —	—	—
2·7	—	5·9	7·5	8·1	11·1	15·0
8·7	—	8·4	9·3	9·9	12·4	15·6
11·1	—	9·3	9·9	9·9	14·3	17·1
13·0	—	9·9	10·5	10·5	14·3	17·1

There appears to be here definite evidence of a gradient in the suction pressure of cells of the beech in proceeding from lower to higher levels. This is obviously of the greatest importance with regard to the questions of transpiration and the ascent of water in trees, questions which, however, cannot be discussed in this place.

Further observations of Ursprung and Blum show that after rain the suction pressure is considerably reduced, as, for instance,.in one observed case of palisade cells, from about 15·6 to 11·1 atmospheres. Also, as with osmotic concentration, there appears to be a daily periodicity in the values of the full suction pressure, there being in general a minimum in the early morning and a maximum in the afternoon.

In later papers Ursprung and Blum (1918 *a*, 1918 *b*, 1919) have extended their observations to other plants, and have in general confirmed the results of their earlier work. Thus in ivy they found that the suction pressure increased with distance from the absorbing zone of the root in any particular tissue, while in a cross-section through an organ the suction pressure increased with distance from the water conducting tissue. The only exception to this rule was found in the absorbing zone of the root, in which, as might be expected, the suction pressure is greater in the cortex than in the piliferous layer.

In a more recent paper (1921) these same authors pay particular attention to the absorbing zone of the root in *Phaseolus vulgaris* and *Vicia Faba*. They find that the suction pressure increases from the piliferous layer inwards as far as the innermost layer of the cortex outside the endodermis, but that the suction pressure of the latter is considerably less than that of the cortical cells outside it, and higher than that of the pericycle within. They regard this anomaly as most probably explained by the presence of different suction pressures at different points on the surfaces of the cells of the endodermis (cf. the last section of this chapter). The suction pressure as measured is the mean suction pressure in cases where the suction pressure varies over the surface of the cell, and if the suction pressure on the outer side of the endodermal cells should be higher than that of the neighbouring cortical cells, and on the inner side lower than that of the adjoining pericycle cells, the entry of water into the central cylinder can be explained.

As regards the value of the suction pressure in other cases, Stiles and Jørgensen (1917 *b*) have shown that a sample of potato tuber neither gained nor lost weight in a $M/4$ solution of sucrose, which indicates a full suction pressure of about 6·5 atmospheres. An experiment with carrot root indicated a suction pressure of about 17 atmospheres. The suction pressure in these cases depends obviously on the previous history of the experimental material, and especially on the degree of desiccation.   .

The exudation of liquid water or a solution from superficial cells of a plant on to the outside surface of the cells takes place in certain cases, as in water pores (water stomata) and nectaries. What appears to be an essentially similar thing is met with in the bleeding of cut plants and in the phenomenon of root pressure, except that in the latter exudation takes place into dead xylem elements instead of on to the surface of the plant.

Several explanations of this exudation of liquid from plant cells have been offered by Pfeffer and subsequent workers, but what is the true explanation must be regarded as still remaining in doubt.

The cells immediately concerned are the exuding cells themselves with an osmotic concentration C_1 corresponding to an osmotic pressure P_1, and the cells internal to, and bordering on, these with an osmotic concentration C_2 corresponding to an osmotic pressure P_2. The suction pressures of the exuding and neighbouring cells respectively are S_1 and S_2. Then if $S_1 > S_2$ water is absorbed from the neighbouring cells. This will proceed until the increased turgor of the excreting cells reduces S_1 until water is no longer absorbed. But if the outer wall of a superficial cell is permeable to water, water will continually pass through this wall on account of evaporation if for no other reason, so that S_1 will be continually increased and there will be in consequence a continual passage of water into the excreting cells from the neighbouring ones. The difficulty is not to account for the movement of water, but for a movement of water sufficiently rapid to bring about an exudation of liquid.

One theory of the mechanism of the exudation of water supposes the protoplasm is more permeable to dissolved substances on the side towards the pore (or xylem vessel) than on the side bordering on neighbouring cells. This was supposed to be the case by Lepeschkin (1906) in the exudation of water by the hydathodes of *Phaseolus multiflorus*, and has been applied by Priestley (1920) to explain the existence of root pressure. It is not proposed to deal here in detail with Lepeschkin's theory of water excretion, for it involves fallacies which have already been noted in these pages and in other writings; it may, however, be worth while to consider briefly the theory of differential permeability of the different parts of the protoplasm (or plasma-membrane) of exuding cells. Let us suppose we have the extreme case in which the protoplasmic membrane is completely impermeable to solutes on the side of the neighbouring cells, but is

somewhat permeable to solutes on the side towards the pore or xylem vessel. Using the same nomenclature as before and assuming the suction pressure is uniform over the whole surface of a cell, if $S_1 > S_2$ water will enter the exuding cells and will only cease to do so when $S_1 = S_2$. But in the meantime solutes are diffusing out from the exuding cells into the pore and the liquid in the pore will thus develop a suction pressure of S_0 equal to the osmotic pressure P_0, since the turgor pressure is zero. This suction pressure will continue to increase until no further exosmosis of osmotically active solutes takes place from the exuding cells, while at the same time the suction pressure of the exuding cells is decreasing. A time will therefore arrive when S_0 ($= P_0$) increases to a value greater than S_1 ($= P_1 - T_1$), at which point exudation of water will take place. If this is the mechanism of water exudation the conditions for this to take place are thus $P_0 > (P_1 - T_1) > (P_2 - T_2)$.

One explanation offered by Pfeffer was to suppose that the exuding cell possessed a higher osmotic concentration in that part of it bordering on the neighbouring cells than in the part adjacent to the water pore (or water conducting element). The suction pressure will thus be different in different parts of the cell. We can then understand that water might be absorbed by the exuding cell both from the neighbouring cell and the pore. This will result in increased turgor of the exuding cell and consequently the wall pressure will soon balance the osmotic pressure on the side of the pore where the osmotic concentration is low, and absorption of water from the pore will stop. Owing to the higher suction pressure on the side of the neighbouring cell, however, water will continue to enter the exuding cell on that side, the turgor pressure will be further increased and in consequence water will be forced out from the exuding cells into the pore or xylem vessel. As V. H. Blackman (1921) rightly points out, if this process is to continue, there must be some mechanism in the cell to prevent mixing of the solutes in the exuding cells and to maintain the difference in concentration in different parts of the cell.

Ursprung and Blum (1921), as already mentioned, consider that a non-homogeneous suction pressure is exerted over the surface of endodermal cells in the absorbing zone of the root, and they suppose that the same may be the case with the parenchymatous cells bordering on the water-conducting elements. They relate the difference in suction pressure in different parts of the same cell rather to differences in the quantity of water imbibed in the cell wall than to differences in osmotic concentration in the cell as suggested by Pfeffer.

INADEQUACY OF THE SIMPLE OSMOTIC VIEW OF THE PLANT
,   CELL AS REGARDS ITS WATER RELATIONS

It will be observed that on the simple osmotic view of the plant cell it is assumed that the amount of water contained in the cell wall and the protoplasm undergoes no change with varying conditions, and that the only interchange of water takes place between the vacuole and the external medium. And even when the view is slightly modified so that the semi-permeable membrane separating the external medium from the osmotically active cell sap is regarded as a thin limiting layer of the protoplasm instead of the whole of it, changes in the water content of the cell wall are neglected, while it is assumed that the protoplasm and the vacuole form one uniform solution as regards osmotic properties. It is also assumed that the swelling or shrinkage of the vacuole is solely to be accounted for on simple osmotic grounds. It is clear that we have no right to make these assumptions without good evidence, so that these questions deserve some little consideration.

In this connexion the work of F. E. Lloyd cited in Chapter VIII is of particular interest as indicating the possibility of explaining the water relations of certain plant cells without the supposition of a surrounding plasma-membrane. Some recent observations of Lapique (1921) are also significant. This writer found that when filaments of *Cladophora glomerata* and *C. oligoclona* are immersed in acid solutions the cells become plasmolysed while the cellulose wall swells up and loses its sharp delimitation. The changes appear to be independent of the osmotic concentration of the external solution as they are observed with $N/1000$ hydrochloric acid as well as with $N/10$. Although alkalies appear to have no influence on the normal cell, yet they act on a cell treated with acid in the reverse sense to the acid. Lapique concludes from these observations that the acidity or alkalinity of the external medium is of more importance for the intake of water than the osmotic concentration of the medium. He further supposes that the cellulose wall and the protoplasm constitute colloidal systems in which the particles carry different charges, as one is coagulated by acids and the other by alkalies.

WATER RELATIONS OF THE CELL WALL

It is usually assumed that the cell wall is saturated with water and that this being so, the amount of water present in the cell wall is unaffected by conditions within very wide limits. It has, however, been shown earlier that the cell wall is not simple in chemical composition

and that it may even be complicated in structure. On this account it is to be regarded as quite possible that under different conditions of stretching or with variations of other conditions, the amount of water held by the cell wall may vary. That cell walls might vary as regards the amount of water contained in them appears to have been fully recognised by Pfeffer (1903).

Some experiments of Hansteen-Cranner (1914) appear particularly suggestive in this connexion. Pith from the leaf stalks of turnips was crushed in a porcelain mortar with water, and after standing for several hours, the solid material was washed repeatedly with water and then re-crushed until no intact cells remained. In this way material was prepared which was regarded as consisting entirely of cell wall. A weighed quantity of this product was dispersed in a very fine condition through cold tap water and allowed to settle on filter paper placed on a sieve. The paper was then removed and dried under pressure in a press. In this way membranes were obtained 90 to 100 μ thick, and used with an area of 3 × 1 cms. Experiments were conducted on the water uptake of these membranes and their behaviour in this respect compared with that of similar membranes from which lipoid substances had been removed by treatment with hot alcoholic hydrochloric acid, and also with membranes of parchment paper.

Such membranes were immersed for definite times in solutions of various salts, and the water absorbed measured by the gain in weight. Measurements were also made of the rate of water loss by evaporation from the membranes after removal from the solutions. In Table XXV are shown some of the results obtained by Hansteen-Cranner with regard to the uptake of water by his artificial cell membranes when immersed in various solutions at 15·5° C., 10 c.c. of liquid being used in each case.

TABLE XXV

Uptake of water by artificial cell-wall membranes in different solutions. (Data from Hansteen-Cranner)

Solution	Concentration in gram-mols. per litre	Uptake of water in percentage of original weight of membrane	Loss of water from soaked membrane by evaporation in air at 13° C. for 10 mins.
CaCl$_2$	0·1 M	152·27	77·61
KCl	0·1 M	205·19	44·77
NaCl	0·1 M	222·00	54·05
Water	—	175·00	62·50
CaCl$_2$	0·01 M	175·82	73·75
KCl	0·01 M	294·87	59·13
NaCl	0·01 M	333·34	62·73
Water	—	172·41	61·00

These numbers indicate clearly that the artificial cell membranes take up less water from solutions of calcium chloride than from equimolecular solutions of potassium and sodium chlorides. The absorption of water from a 0·2 M solution of calcium nitrate was also less than that from an equimolecular solution of potassium nitrate. Conversely, the water taken up by the membrane in calcium salts appears more easily lost by evaporation than that taken up by the membranes immersed in potassium and sodium salts. As Hansteen-Cranner found exactly the same relation to hold between the uptake of water and loss of water by evaporation by whole plants with their roots in solutions of salts of the same metals, he concludes that living cell walls are responsible for this difference in water uptake from different salts. The numbers given in Table XXVI, compared with those in the previous table, indicate the similarity in the behaviour of whole plants and artificial membranes.

TABLE XXVI

Uptake of water by oat plants growing in culture solutions of different salts. (Data from Hansteen-Cranner)

Solution	Relative water uptake per gm. of roots	Relative water loss per gm. of transpiring part
$N/100$ KNO$_3$	100	100
$N/100$ NaNO$_3$	86·34	100·24
0·177 % Ca(NO$_3$)$_2$ (isosmotic with $N/100$ KNO$_3$)	82·01	100·90
$N/100$ KNO$_3$	100	100
$N/100$ NaNO$_3$	82·48	77·64
$N/100$ Ca(NO$_3$)$_2$	76·43	103·91
0·177 % Ca(NO$_3$)$_2$	82·05	113·89

It is to be noted that parchment paper membranes do not behave in the same way as the artificial cell wall membranes, for the uptake of water by parchment paper was almost the same whatever the salt dissolved in the water. Obviously parchment paper membranes cannot be compared physiologically with cell walls. On the other hand, lipoid-free artificial cell wall membranes behave in a similar fashion to the lipoid-containing membranes as regards uptake of water from solutions, even absorbing more water than the untreated membranes, while the parchment paper membranes take up considerably less.

These results suggest strongly that the uptake of water by the cell wall is influenced by the nature of the solutes in the external medium, so that the cell wall cannot be neglected in a complete view

of the water relations of the plant cell. Of course, where the volume of the cell wall is very small in comparison with that of the vacuole the differences in the absorption of water by the cell wall under different conditions may be negligible; in other cases the cell wall must obviously be taken into account.

WATER RELATIONS OF THE PROTOPLASM

Just as the cell wall is capable of absorbing water by imbibition, so is the protoplasm. Indeed, Pfeffer (1900) states that "the cell-wall, protoplasm, and indeed all organized structures have the power of imbibing water and swelling." Experiments with non-living systems such as gelatin, agar-agar, proteins and mixtures of these and other substances, strongly suggest that the capacity of the proto-plasm for absorbing water is highly dependent on the concentration and nature of the solution with which it is in contact. Acids and alkalies appear to exert the greatest influence on the power of such systems to absorb water, both increasing the swelling capacity considerably. Very comparable results have been obtained by Loeb (1897, 1898), Fischer (1910) and Miss D. J. Lloyd (1916) with animal tissues such as frog muscle and sheep's eyes. Whether such phenomena are capable of explaining completely the water relations of the non-vacuolate cell without the invocation of a limiting semipermeable membrane to the protoplasm is a question which has been discussed earlier without our being able to come to any definite conclusion. Further evidence from experiments with plants is badly needed. But that protoplasm has such powers of swelling there can be no doubt, nor can it be doubted that the degree of swelling will be influenced by the solution exterior to it. Substances may also be present in the vacuole to which similar considerations apply. Where the volume of the protoplasm is very small in comparison with that of the vacuole, and where the swelling capacity also is not great, such swelling of the protoplasm can be neglected in comparison with the water exchange due to the simple osmotic relations of the cell. In other cases the swelling of the protoplasm may be an important factor in determining the water relations of the plant cell. This appears to be the case, for instance, in many succulent plants such as the cacti, in which the presence in the cell of pentosan mucilages may render the swelling of the cell contents the determining factor in the passage of water between the cell and the exterior. Similar swelling occurs in red algæ (McGee, 1918).

THE EFFECT OF PERMEABILITY OF THE PROTOPLASM TO SOLUTES ON THE WATER RELATIONS OF THE CELL

In considering the water relations of the cell on the simple osmotic view it has been assumed that the protoplasm is impermeable to the solutes contained in the cell sap, and also to the solutes present in the external liquid. It is further assumed that the external medium exerts no influence on the permeability of the protoplasm. Where these assumptions do not hold our considerations have to be modified.

In the first case let it be supposed that a proportion of the solutes in the cell sap are capable of passing through the protoplasm. These solutes will then diffuse through the protoplasm until the concentrations of each particular solute within and without the protoplasmic membrane are in equilibrium with one another. This will mean that the osmotic concentration of the cell sap is probably lessened so that in consequence the cell would take up less water, or lose more, than it would if the protoplasm were completely impermeable to all the solutes in the vacuole. On the other hand, if there are substances in the liquid external to the cell which can penetrate the protoplasm, the cell, when equilibrium is reached, will contain more water than it otherwise would, since the osmotic concentration is raised. Probably in most cases both phenomena occur, although the former may take place to a very limited extent. Some substances, such as ethyl alcohol, ether, acetone and chloral hydrate, may penetrate the protoplasm so rapidly that plasmolysis is not produced by hypertonic solutions (Overton, 1895). Should the substance external to the cell influence the permeability of the protoplasm, as, for instance, by adsorption or chemical action, so that less or more solute passes through the protoplasmic membrane than otherwise would, the water relations of the cell will be correspondingly altered.

THE EFFECT OF IMPERFECT PERMEABILITY OF THE CELL WALL TO SOLUTES ON THE WATER RELATIONS OF THE CELL

It is generally assumed on the simple osmotic view of the cell that the cellulose cell wall is completely permeable to most dissolved substances, at any rate, such crystalloidal substances as acids, alkalies, salts and sugars. Apart from the well-known cases of semi-permeable cell walls which have been dealt with in Chapter VII, it is by no means certain that cellulose walls are always as permeable to dissolved substances as to water. Indications of an imperfect permeability of the cell walls for sugar molecules were observed by Wächter (1905) in beet root and onion bulb scales and by

Miss Delf (1916) in onion leaves. How general such a complication may be there is no evidence to tell; it is a possibility which should not be lost sight of in dealing with problems connected with the relations of the cell to solutions.

Where such a complication is present the course of plasmolytic shrinkage will obviously differ from the normal case where the cell wall is as completely permeable to the solute as to water. The rate of separation of the protoplast from the cell wall will take place more slowly, as it will take time for the solution outside the protoplast to reach the plasmolysing concentration.

The Effect of Tissue Tensions on the Water Relations of Cells Enclosed in Tissues

That different cells in the same organ may be subjected to different external pressures from surrounding cells and tissues is indicated by the changes in shape which pieces of tissue may undergo when isolated from the intact plant body. Thus the curvatures of longitudinal strips of dandelion scapes mentioned in an earlier section of this chapter indicate that in the intact scape the central tissue must have been in a state of compression, for it elongates on isolation of the strip, while the epidermal tissue contracts, showing that this tissue was stretched and under tension. Investigations on tissue strains have been numerous, but a discussion of this subject does not lie within the scope of this work. For general information on the subject the reader is referred to Chapter V of the second volume of the English edition of Pfeffer's *Physiology of Plants* (1903). But the presence of these strains must influence very materially the water relations of the cells in tissues where such strains exist. Yet as far as I know this question is only discussed in one place, namely, in the excellent paper by Höfler (1920) on the osmotic relations of the plant cell. Höfler says himself that the subject has never been investigated either theoretically or experimentally. The relations are obviously complex. Let us consider a tissue which has been immersed in a hypertonic solution, or which has lost water by evaporation, so that all the cells within it are turgorless. If such a tissue is now immersed in water, the latter enters at first on all sides so that neighbouring cells as they swell press against one another. The only space then into which the cells can stretch further is the intercellular space system. Hence the cells become deformed with a non-uniform stretching of the cell wall, and consequently an uneven distribution of the wall pressure. A state of equilibrium is still possible under

these conditions because the pressure balancing the hydrostatic pressure of the cell, the turgor pressure, which must be uniform throughout, is made up of the sum of the wall pressure and a component of the pressure exerted by neighbouring cells, which may vary at different points on the wall of the cell under consideration, since a number of cells are concerned, namely, all the neighbouring cells. Höfler supposes that under these conditions water saturation is reached sooner than it would be in the isolated cell because in addition to the inwardly directed pressure of the stretched wall of the cell itself there is the pressure exerted by the neighbouring cells to prevent further increase of the volume of the cell. If this view is correct, Höfler points out a very significant consequence of it in reference to the suction pressure. As with progressive intake of water the turgor pressure must increase to its maximum more rapidly than it would if isolated, it follows that the suction pressure must increase more rapidly with decreasing turgidity in the case of the cell in a tissue than in the isolated cell, other things being equal, since the suction pressure is equal to the osmotic pressure less the turgor pressure. That is, the suction pressure can vary greatly within a small range of turgidity. This may be of great importance from an ecological point of view.

CHAPTER X

THE INFLUENCE OF EXTERNAL CONDITIONS ON THE INTAKE AND EXCRETION OF WATER BY PLANT CELLS AND TISSUES

THE discussion of the water relations of the plant cell in Chapter IX involved a consideration of the internal conditions which may influence the rate of passage of water through the cell wall and protoplasm. These conditions are the concentration and nature of the substance dissolved in the cell sap which between them determine the osmotic pressure of the cell. The permeability of the protoplasm to solutes will also influence the rate of intake of water, inasmuch as the passage of solutes into or out from the cell will tend respectively to raise or to lower the osmotic concentration of the cell sap and hence the rate of intake of water through the protoplasm and cell wall. Lepeschkin (1908 a) appears to suggest that the osmotic pressure of a solution is a function of the permeability of the membrane, but the error of this view is obvious and has already been indicated by Brooks (1917 a), Höfler (1920) and V. H. Blackman (1921). Apart from electrical effects, the osmotic pressure which can be developed with a permeable membrane must continually fall with time until equilibrium of the penetrating solute on the two sides of the membrane is attained.

External conditions which are known to influence the rate of passage of water into and out from the cell are temperature and the composition of the external medium. It has to be noted that changes in the rate of passage of water into and out from the cell are not necessarily to be attributed to changes in the permeability of the protoplasm. For, neglecting the possible effect of limiting plasma-membranes, the rate of intake or excretion of water is the resultant

of (1) effects attributable to purely osmotic relations of the cell, (2) effects due to the entrance or exosmosis of dissolved substances which alter the osmotic relations, (3) effects on the capacity for imbibitional swelling of cell wall, protoplasm and vacuole, and (4) effects resulting from any influence on the permeability of the cell wall and protoplasm. It may not always be possible to distinguish with certainty which of these various effects is or are mainly responsible for observed behaviour. It will therefore be most satisfactory first to describe the results obtained experimentally, and then to discuss how far these observed results are to be correlated with protoplasmic permeability or with other phenomena indicated above.

Temperature

A number of earlier observations have suggested that the rate of intake of water by, or its excretion from, plant cells and tissues, is considerably influenced by temperature. Among these may be mentioned those of Vesque (1877) on the absorption of water by forest trees, Wieler (1893) on the bleeding of cut stems of a number of plants, and Lepeschkin (1906) on the exudation of water by the sporangiophores of Pilobolus. As these results, suggestive as they are, do not provide us with exact data for our analysis, we may pass at once to a consideration of those researches in which exact measurements of the effect of temperature on water intake or excretion have been attempted.

DETERMINATIONS BY THE PLASMOLYTIC METHOD

Systematic attempts to determine the influence of temperature on the rate of passage of water through the cell membranes were made by Krabbe (1896) and van Rysselberghe (1901). The former measured the time required for complete contraction of cylinders of turgid pith when plasmolysed at different temperatures and concluded that the rate of excretion and absorption of water rises with increase of temperature.

Van Rysselberghe used cylinders of elder pith, epidermal cells of *Tradescantia* and *Begonia*, *Lemna*, *Spirogyra* and other plants. The cylinders of pith were plasmolysed at different temperatures in strong sucrose solutions ($0.731\ M$) and the amount of shrinkage measured at different times over a period of 24 hours. The temperature effect was determined by comparing the amount of shrinkage after two hours at the different temperatures. But obviously this

could only give a correct indication of the temperature effect if the relative amounts of shrinkage at the different temperatures were the same at all times during the experiment, which was not the case. Had he chosen to compare the shrinkage after three or four hours instead of after two, he would have obtained a quite different set of values for the relation between temperature and rate of passage of water through the cell membranes. The only way to obtain correct information on the influence of temperature in these experiments is to compare the *rate* of passage of water out of the tissues at different temperatures, but under otherwise exactly the same conditions. This is to be accomplished, as pointed out by Miss Delf (1916), by constructing the time-shrinkage curves for the different temperatures and then comparing the rate of shrinkage *at the same stage of shrinkage*, as given by the value of tan α, where α is the angle made by the tangent to the curve with the time axis.

The data of van Rysselberghe have been subjected to this treatment by Miss Delf, when they indicate an increase in the rate of passage of water through the cell membranes with increasing temperature up to about 20° C., but between this and 30° C. the effect of temperature is negligible. Miss Delf found that such a result is obtainable only when a very strong plasmolysing solution is used, the reason being apparently that there is an "upper limit to the rate at which the mechanical tissue system of cells can collapse and shrink," so that with the strong solutions used by van Rysselberghe a great part of the contraction curves obtainable from his data represent a contraction of the cell wall system over which permeability has no control.

Other experiments of van Rysselberghe, in which only the times taken for cells to plasmolyse or deplasmolyse are given, are obviously open to still greater objection, both on the grounds already stated in the case of the experiments with pith, and also on account of the difficulty of carrying out such experiments with exactitude. Further, it is doubtful whether sufficient account has been taken of the great variability of plant tissue from different individuals of the same species, so that it is not certain whether van Rysselberghe's results could be accepted as expressing more than approximately the relation between temperature and rate of intake or excretion of water, even · if they were not open to grave objection on other grounds. No further consideration will therefore be given to the results of Krabbe and van Rysselberghe in this place.

Determinations of the Effect of Temperature by the Method of the Rate of Linear Shrinkage of Turgid Tissues

The first critical research on the influence of temperature on cell permeability to water is that of Miss Delf (1916). Her method was similar in principle to that used by van Rysselberghe, but was considerably more sensitive. By means of the optical lever, which had already been introduced into plant physiology by Bose (1906) to record small movements in plants, changes in length of short strips of tissue could be observed under a magnification of 350 diameters. The apparatus devised by F. F. Blackman and used by Miss Delf is directly applicable only to the investigation of hollow cylindrical structures, those used by Miss Delf being onion leaves and dandelion scapes.

The essentials of the method are as follows. The lower end of the plant cylinder is firmly fixed by means of cotton to a glass tube of narrow bore which passes through a cork held immovable by a metal clamp. The upper end of the cylinder is pierced by a fine glass hook attached to a loop of very fine waxed cotton which at its upper end is itself attached to the projecting wire arm of the optical lever, which carries a small mirror reflecting the image of an illuminated cross wire on to a millimetre scale. Contraction of the cylinder can thus be very accurately measured by the movement of the image of the cross wire on the scale. For details of the apparatus and procedure, including the method adopted for obtaining different constant temperatures, reference must be made to the original paper.

The material selected for investigation already noted above was chosen because the inner surface of the hollow cylinders of which it is composed is lined entirely with cells possessing thin cellulose walls. Solutions of cane sugar in various concentrations (0·18, 0·3, 0·5 and 0·731 weight-molecular solutions) were allowed to flow through the hollow cylinders of plant tissue and the contraction resulting followed by the optical lever arrangement. The total contraction produced by these solutions varies somewhat with the age of the material, but very greatly with the concentration of the solution. Thus dandelion scapes in 0·30 M sucrose contracted to the extent of 0·2 to 0·4 per cent. of the original length, while in 0·731 M sucrose the contraction was as much as 2 to 2·5 per cent. of the original length. Similarly, onion leaves in 0·18 M sucrose contracted 0·3 to 0·5 per cent. and in 0·731 M sucrose 3 per cent.

of the initial length. Although greater contractions were obtained in the stronger sucrose solutions, it was found almost impossible to decide how much of this was to be attributed to plasmolysis, and how much to the after-shrinkage of the cell walls. On this account weaker solutions, "subtonic," that is, diluter than isotonic, were used for the crucial experiments, the actual concentrations chosen being such that the contractions produced after the tissue had been allowed to swell to equilibrium in pure water were sufficiently large to be measured satisfactorily with the apparatus employed, while a definite end-point was reached in about two hours. The solutions which fulfilled these conditions were 0·18 M sucrose for onion leaves and 0·3 M for dandelion scapes.

With the subtonic solutions employed the shrinkage was found to follow an approximately logarithmic course. Miss Delf states that "with great care in the choice of material and in repeating the same experimental conditions, there was a certain amount of variation in the rate of plasmolysis at the same temperature. At medium temperatures, therefore, it was usual to perform several experiments under the same conditions, and to select the ones which gave the curve of approximately logarithmic form." This selection of certain curves appears a weakness in Miss Delf's work, for as the rejected experiments were conducted with the same care and under the same conditions as the selected experiments, the form of the time-shrinkage curves does not seem an adequate ground for selecting some and rejecting others.

At temperatures above that of the laboratory complications occur. These are (1) a contraction resulting from the effect of prolonged high temperature, probably on account of the escape of water from the cells as a result of increased exosmosis of solutes from the cell sap at high temperatures, and (2) an expansion resulting probably from the entry of sucrose into the cell. Up to 35° C. in the case of the dandelion scape, and up to 36° C. in the onion leaf, it was found to be unnecessary to apply any correction on account of these complications, but above this temperature the shrinkage due simply to the presence of the subtonic solution at the higher temperature was obtained by subtracting the shrinkage resulting in distilled water at the higher temperature from the total shrinkage actually observed.

From the shrinkage-time curves obtained by Miss Delf the rates of contraction at 30, 50 and 70 per cent. of the total contraction were calculated for the temperatures employed in the experiments, and the rates for temperatures between 5° and 40° C. found by inter-

polation. The relative values for the rate of linear shrinkage of the tissue at the stage of half-contraction are shown in Table XXVII, the values for 25° C. being taken as unity.

TABLE XXVII

Relative rates of linear contraction of onion leaves and dandelion scapes in subtonic solutions of sucrose at different temperatures.
(Data from Miss Delf)

Temperature in centigrade degrees	Relative rate of linear contraction	
	Onion leaves in 0·18 M sucrose	Dandelion scapes in 0·3 M sucrose
5	0·36	—
10	0·44	0·22
15	0·50	0·30
20	0·66	0·50
25	1·0	1·0
30	1·7	1·9
35	2·9	3·0
40	5·0	5·0

From these numbers the temperature coefficients (Q_{10}, see Chapter III, p. 101) for a rise of 10° C. were calculated, with the results given in Table XXVIII.

TABLE XXVIII

Temperature coefficients for rate of linear shrinkage of onion leaves and dandelion scapes in subtonic sucrose solutions.
(Data from Miss Delf)

Range of temperature in centigrade degrees	Temperature coefficient (Q_{10})	
	Onion leaves	Dandelion scapes
5–15	1·4	—
10–20	1·5	2·3
15–25	2·0	3·3
20–30	2·6	3·8
25–35	2·9	3·0
30–40	3·0	2·6

It is particularly interesting to consider how far these temperature effects are to be regarded as effects of temperature on permeability of the protoplasm, as they are assumed to be by Miss Delf.

If the permeability of the cell or tissue to water is defined as the quantity of water passing through unit area of the cell membranes (cell wall + protoplasm) in unit time under unit difference of pressure we have the equation

$$\frac{dw}{dt} = \zeta \, (P_e - P + T) \, A,$$

where $\dfrac{dw}{dt}$ is the rate of passage of water out of the cell or tissue, ζ is the permeability, P_e and P are the osmotic pressures of the external solution and the cell sap respectively, T the turgor pressure (cf. Chapter IX), and A is the area of the cell surface through which water passes.

Now the rate at which water is lost by the tissue is proportional to the rate of volume shrinkage, and if c.g.s. units are employed may be taken as equal to it. Let us suppose that in a small time interval δt the length l of the cylinder contracts by a small length δl, and that in the same time the contraction in width is δa, the original diameter of the cylinder being r and the thickness of the tissue a. Let us further suppose the water loss in the same time is δw. The change in volume in the time δt is then

$$\pi a l\, (2r - a) - \pi\, (a - \delta a)\, (l - \delta l)\, \{2\, (r - \delta a) - (a - \delta a)\}$$

or $\quad \pi\, [\{a\, (2r - a)\, \delta l + 2rl\delta a\} - (l\delta a + 2r\delta l + \delta a\delta l)\, \delta a].$

Hence, using c.g.s. units, we have in the limit the rate of water loss by the tissue given by

$$\frac{dw}{dt} = \pi a\, (2r - a)\, \frac{dl}{dt} + 2\pi rl\, \frac{da}{dt}.$$

Whence $\quad \zeta = \dfrac{\pi}{P_e - P + T} \left\{ a\, (2r - a)\, \dfrac{dl}{dt} + rl\, \dfrac{da}{dt} \right\} \dfrac{1}{A}.$

Thus, if it is assumed (1) that differences in imbibitional swelling at different temperatures are negligible, (2) that the value of the exudation pressure $P_e - P + T$ at the same stage of shrinkage is practically constant for temperatures over the range $5°$ to $42°$ C., and (3) that the cylinders shrink only in a longitudinal direction, the above equation may be written

$$\zeta = K\frac{dl}{dt},$$

where K is a constant since A is the same for all cylinders in the same stage of shrinkage. If, on the other hand, there should be a shrinkage in a transverse direction proportional to the longitudinal shrinkage, the equation connecting permeability and shrinkage may be written

$$\zeta = Kl^2 \cdot \frac{dl}{dt},$$

K again being a constant.

Now the first two assumptions are very reasonable, and it is probable that the actual shrinkage would approximate to one of the two cases considered under (3). In the first of these cases the

permeability is proportional to the rate of linear shrinkage, in the second tp the rate of linear shrinkage multiplied by the square of the length. Hence if comparisons are only made,' as in the case of Miss Delf's experiments, with cylinders in the same stage of shrinkage, l^2 will be the same in all cases compared with one another and the permeability will similarly be proportional to the rate of linear shrinkage.

The values obtained by Miss Delf may then legitimately be regarded as measures of the permeability of the cell membranes (cell wall + protoplasm) to water. Miss Delf speaks of them as measures of the permeability of the protoplasm, but as the effects of cell wall and protoplasm are not separated, they are better regarded as measures of the permeability of the cell. It will be noted that errors arising from exosmosis of dissolved substances and intake of salt are allowed for in the experimental procedure, and need not therefore be further considered.

DETERMINATIONS OF THE INFLUENCE OF TEMPERATURE ON THE RATE OF INTAKE OF WATER BY THE CHANGE IN WEIGHT METHOD

Storage Tissues. The influence of temperature on the intake of water by storage tissues, potato tuber and carrot root, has been investigated by Stiles and Jørgensen (1917 *b*). In the method employed by them, the course of absorption of water at different temperatures is followed by immersing the tissue of known weight in water and weighing the tissue at intervals. The change in weight may be fairly attributed to gain or loss of water only, as the weight of dissolved substance which diffuses out from the tissue during the period of the experiment is negligible in comparison with the weight of water involved.

In order to eliminate errors due to the variability of tissue in different samples, a variability which may be very large, and to ensure equality of surface and weight of tissue in all comparative experiments, the following procedure was adopted. Cylinders of tissue were cut from the tubers or roots by means of a cork-borer 1·8 cms. in diameter. These cylinders were then cut into disks of a uniform thickness of 0·2 cm. by means of a hand microtome. For any series of experiments performed for comparative results all the disks required were cut at once, well mixed together and weighed in sets of 20. In this way samples of the material, each sample consisting of 20 disks, were obtained having approximately

the same weight and surface. The experiments at any one tem-
perature were all conducted in triplicate or quadruplicate, so that
for a comparison of the rate of water intake at four different tem-
peratures, 12 or 16 sets of 20 disks were required, 3 or 4 at each
temperature. The probable error of the determinations was calculated
so that some idea of the degree of reliability of the results might be
obtained. The temperatures were maintained constant by means
of thermostat baths provided with gas-mercury-toluene regulators.
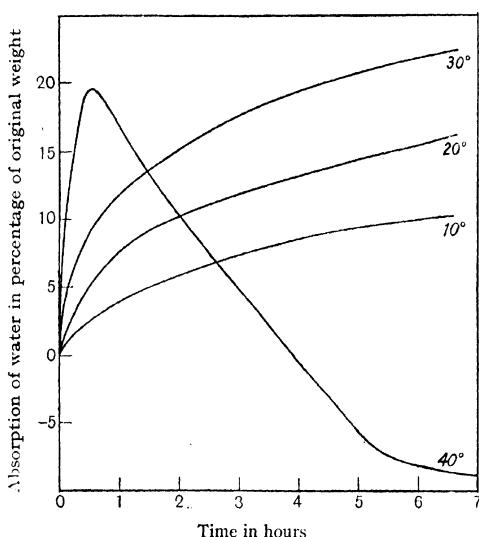
Fig. 7. Curves illustrating the influence of temperature on the absorption
of water by potato tuber. (After Stiles and Jørgensen.)

The course of water absorption at different temperatures by
potato and carrot is shown in Figs. 7 and 8. From these absorption-
time curves the rate of swelling of the tissue has been determined in
the same way as the rate of shrinkage in Miss Delf's experiments,
that is, by measuring the tangent of the angle made with the time
axis by the tangent to the curve at any particular stage of swelling.
By comparing the rate of intake of water at different temperatures
at the same stage of swelling, the following temperature coefficients
for the rate of intake of water are obtained.
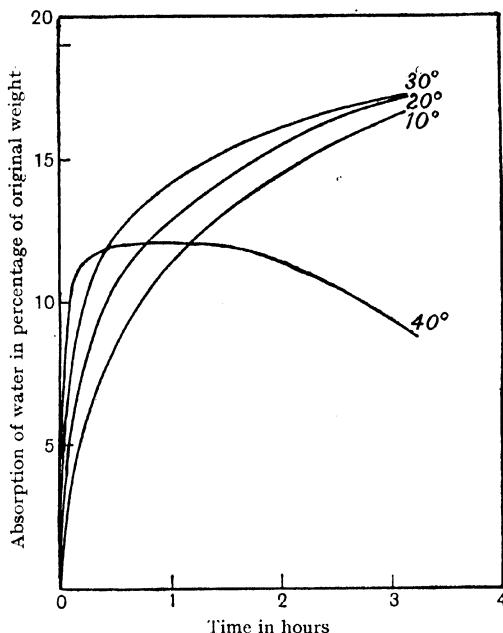
Fig. 8. Curves illustrating the influence of temperature on the absorption of water by carrot root. (After Stiles and Jørgensen.)

TABLE XXIX

Temperature coefficients of the rate of intake of water by storage tissues. (Data from Stiles and Jørgensen)

Temperature range in centigrade degrees	Temperature coefficient (Q_{10})	
	Potato tuber	Carrot root
10–20	3·0	1·3
15–25	2·75	1·4
20–30	2·7	1·6

Two points deserve special notice in regard to the results of Stiles and Jørgensen. In the first place, the rate of swelling of carrot was found to be considerably greater than that of potato. Thus at 10° C. the rate of swelling of carrot was about nine times that of potato under apparently the same conditions with regard to degree of swelling, while at 30° C., in spite of the lower temperature co-efficient in the case of carrot, the rate of swelling was still a little

more than twice as great in carrot as in potato. This is probably due in part to the higher suction pressure observed in the carrot ,tissue used (cf. Chapter IX), but as there is no evidence that the tissues were in the same absolute stage of swelling, too much stress must not be laid on the difference between the two tissues.

In the second place a phenomenon noted by Miss Delf in onion and dandelion appears also in carrot and potato tissue, namely, the contraction and loss in weight of the tissues at temperatures above 30° C., which probably corresponds to a rapid exosmosis of solutes from the cell. This breaking down of the semi-permeability of the protoplasm requires some time to become obvious, so that, as the curves for 40° C. show in both potato and carrot, there is first an initial period of rapid absorption of water followed by exudation. This phenomenon has also been observed in other storage tissues, such as red beetroot and artichoke tubers (*Helianthus tuberosus*).

Confining attention only to the curves where this complication is not present, and using the same notation as in the preceding section, we have the relation

$$\frac{dw}{dt} = \zeta \, (P - T) \, A,$$

where it is assumed as before that the effect of temperature on imbibitional swelling is negligible. If it is also assumed as before that $P - T$ is approximately constant over the range of temperature in question, the temperature coefficients given above may be accepted as those of the permeability of the cells of the tissues to water.

Seeds. The influence of temperature on the absorption of water by barley grains was investigated by A. J. Brown and Worley (1912) by means of the change in weight method. Known weights of grains were immersed in water kept at temperatures of 3·8° C., 21·1° C. and 34·6° C., and the increase in weight determined after various intervals of time. From the absorption-time curves so obtained the rate of water intake at different stages of absorption was measured for the three temperatures employed. The values obtained indicated that the rate of water absorption at 21·1° is about 3·40 times the rate of absorption at 3·8°, while the rate at 34·6° is about 2·44 times that at 21·1°. These numbers give a mean value for the temperature coefficient (Q_{10}) of about 2 (actually 1·99), a value characteristic of a number of chemical reactions. Somewhat lower values were obtained by Shull (1920) in a series of carefully conducted

experiments with seeds of *Xanthium pennsylvanicum* and small Scotch yellow split pea, the values for Q_{10} between 5° and 35° C. found for *Xanthium* being 1·55 in one case, and 1·83 in another, and for pea 1·6. Very similar, though on the whole lower values, were obtained by Denny (1917 *a*) for the temperature coefficient of the rate of passage of water through the detached seed coat of *Arachis hypogaea* (1·34 to 1·64).

Brown and Tinker (1916 *a*) regard the rate of entry of water into barley grains as given by the equation

$$\frac{dy}{dt} = k\,(a - y),$$

or, integrating,

$$\log \frac{a}{a - y} = kt,$$

where y is the quantity of water absorbed in a time t, and a and k are constants. Shull, however, holds that both his own experimental results and those of Brown and Worley are better expressed by the equation

$$y = a \log_{10} (bt + 1) + c,$$

where y is the quantity of water absorbed, t the time of immersion and a, b and c are constants. For the whole course of water intake the time-absorption curves can, in some cases at any rate, best be represented by three such equations representing the early, middle and late stage of water absorption. Apart from their value in enabling the temperature coefficients to be calculated with exactitude, these equations do not help us much in explaining the processes of water intake.

In the case of seeds, where the percentage of water is comparatively low and the percentage of colloidal substances susceptible to imbibitional swelling very high, it is clearly impossible to describe the temperature coefficient of water absorption as a temperature coefficient of the cell membranes. To determine the effect of temperature on the different processes involved in the water intake by seeds, further experimental work is necessary. Denny's work with seed coats separated from the seeds suggests a way in which this might be attempted. Sufficient data are, however, at present lacking.

ON THE VALUES OF THE TEMPERATURE COEFFICIENT OF THE
PERMEABILITY OF THE CELL TO WATER

Three points deserve special mention in regard to the temperature coefficient of the cell membranes to water.

1. The permeability to water increases continuously from the lowest to the highest temperatures investigated. This means that, other factors being equal, water absorption or excretion increases continuously with rise of temperature. Above 30° C. a "time factor" (F. F. Blackman, 1905) appears, inasmuch as with prolonged immersion in water at such higher temperatures the initial absorption is followed by excretion of water; this has been observed in onion leaves, dandelion scapes, tubers of potato and artichoke, and roots of carrot and red beet.

2. The temperature coefficients vary considerably for different tissues and over different temperature intervals in the case of the same tissue, the lowest observed being for carrot root and the highest for dandelion scapes.

3. The temperature coefficients are higher, and often considerably higher, than those found for a physical process such as diffusion of solutes in water, which are generally between 1·2 and 1·3, and approach instead more closely those characteristic of chemical processes. Yet there is no ground for supposing that we have to do here with a chemical rather than a physical process. The results rather suggest the necessity for caution in drawing conclusions with regard to the nature of a process from the value of the temperature coefficient.

Brown and Worley point out that an exponential increase in the value of a physical property is very rare, but that vapour pressure of water is an exception. The general equation

$$v = ae^{k\theta}$$

gives both the relation between vapour pressure and temperature and that between water absorption by barley grains and temperature. Shull, however, does not agree that the rate of intake of water by seeds is determined by vapour pressure. He is no doubt correct in supposing that in the case of seeds the rate of water intake is determined by a number of factors, among which chemical changes in the colloids of the seed coat may not be unimportant. Denny also, on account of the values of the temperature coefficient found by him, disagrees with Brown and Worley in regard to vapour pressure of water determining the rate of entrance of water into seeds.

Dissolved Substances

The investigation of the influence of dissolved substances on the intake and exosmosis of water by plant tissue (potato tuber and carrot root) has also been investigated by Stiles and Jørgensen (1917 *b*) using the change in weight method. Some further observations have been made by Thoday (1918 *a*) using the same method in a cruder form. As the results obtained show that the influence of all dissolved substances is not the same, it will be convenient to consider the different cases separately.
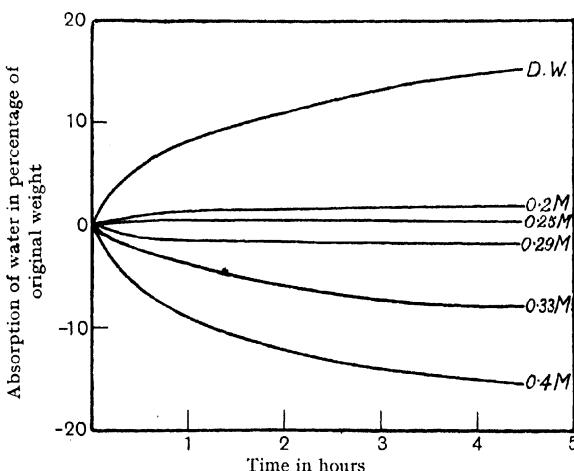
Fig. 9. Curves illustrating the absorption of water by potato tuber immersed in distilled water and sucrose of various concentrations (0·2 *M* to 0·4 *M*). (After Stiles and Jørgensen.)

1. SUCROSE

Experiments were conducted on potato tissue at 20° C. with the same technique as that already described. Sucrose solutions of various concentrations were employed, the absorption-time curves for the tissue in the different concentrations of sucrose being shown in Fig. 9. It will be observed that with increasing concentration of sucrose both the rate of water intake and the total amount of water absorbed are reduced, and that in a solution of about *M*/4 concentration the intake of water is approximately zero. With higher concentrations of the sugar exosmosis of water takes place, both the

rate of exosmosis and the quantity of water exuded increasing with increase of sugar concentration.

* Since, as we have already noted, the rate of intake of water is proportional both to the permeability and to the net suction pressure, so that

$$\frac{dw}{dt} = \zeta (P - P_e - T) A,$$
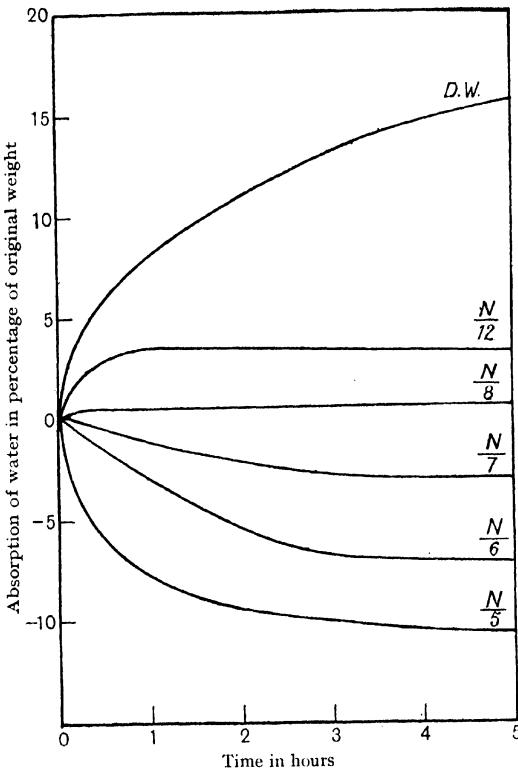
Fig. 10. Curves illustrating the absorption of water by potato tuber immersed in distilled water (*D.W.*) and sodium chloride solutions of various concentrations $\left(\frac{N}{5} \text{ to } \frac{N}{12}\right)$. (After Stiles and Jørgensen.)

and as with increase in the concentration of the sugar P_e increases, and consequently $P - P_e - T$ decreases, the observed results can be explained on account of the reduction of the net suction pressure with increasing concentration of the external sugar solution; there

is no evidence from existing data that the presence of the sugar has any influence on the permeability of the cell membranes to water, nor is there any evidence of an appreciable entrance of the sugar nor of exosmosis of solutes from the cell sap. Similar results with potato in sucrose solutions were obtained by Thoday (1918 *a*).

2. SODIUM CHLORIDE

Similar experiments with sodium chloride showed that this salt behaved to potato tissue in the same way as sucrose, but that the solution in which the tissue neither gained nor lost in weight possessed a concentration slightly higher than $N/8$. This lower concentration is to be expected from theoretical considerations as the ionisation of the sodium chloride in solution almost doubles its osmotic pressure. The absorption-time curves for the tissue in different concentrations of the salt are shown in Fig. 10.

Similar experiments with carrot tissue showed that in a solution of $N/3$ concentration this tissue neither gained nor lost in weight.

3. ETHYL ALCOHOL, OCTYL ALCOHOL, CHLOROFORM AND MERCURIC CYANIDE

The results obtained with ethyl alcohol were quite different from those with sucrose and sodium chloride. The results with potato tissue are shown graphically in Fig. 11. With lower concentrations (up to molecular) absorption at first takes place as in dilute solutions of sucrose. But it will be observed that if ethyl alcohol behaved similarly to sucrose, we should expect the potato to undergo neither gain nor loss in weight in a solution having a concentration of about $M/4$, whereas swelling even takes place initially in solutions having as high a concentration as $2M$. But in concentrations M and $2M$ this swelling reaches a maximum, after which the solution loses water. With $M/2$ alcohol the maximum was not reached even after 16 hours, but the swelling was lower than in distilled water; in M alcohol the maximum intake of water is reached in about 7 or 8 hours, while in $2M$ alcohol in about half an hour. With concentrations of alcohol higher than $2M$ any preliminary period of water intake was not detected by the method, the excretion of water being rapid, the more so the higher the concentration of the external solution. The observation of Overton (1895) that ethyl alcohol may enter the cell so rapidly from hypertonic solutions that plasmolysis does not take place is in agreement with the behaviour noted here.

The behaviour of a secondary octyl alcohol was similar, and the curves given by Thoday for chloroform and mercuric cyanide leave ⏺little doubt that these substances behave on the whole similarly to the alcohols examined.

These results are simply explained. It is clear that these substances bring about an increase in the permeability of the cell to substances dissolved in the cell sap (Czapek, 1910 *a*; Stiles and Jørgensen, 1917 *a*), while at the same time it is to be supposed that the solute in the external liquid is itself capable of entering the cell.
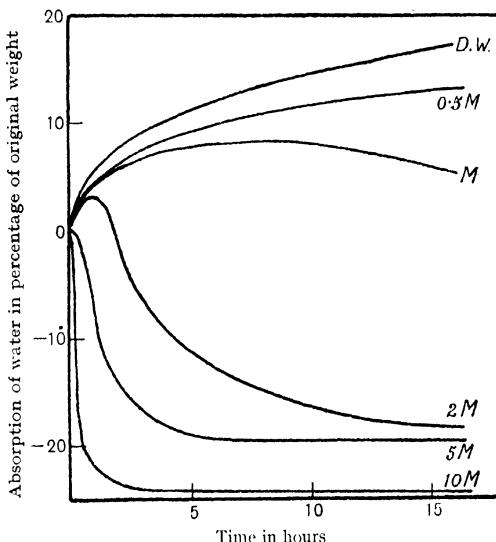
Fig. 11. Curves illustrating the influence of ethyl alcohol in concentrations 10 *M* to 0·5 *M* on the absorption of water by potato tissue. (After Stiles and Jørgensen.)

In the first stage of the experiment there will thus occur a rise in the osmotic pressure of the cell-sap on account of the addition to it of alcohol or other substance. If the permeability of the cell membranes remained unaltered we may suppose that the swelling in the most concentrated of the alcoholic solutions would be the same as that in distilled water at equilibrium, but the rendering permeable of the cell to the solutes in the cell sap brings about a diminution of osmotic pressure within the cell which accordingly loses water on account of this. Thus to the first stage of excessive swelling there follows a

stage of water loss as the membranes are rendered more permeable. The exact shape of the absorption-time curve in any particular case must depend on the concentration of the external solution, the permeability of the cell to the external solute and the rate at which the solute acts on the cell membranes in rendering them permeable to the solutes of the cell sap. Thus it is not to be expected that the behaviour of the tissue in regard to water gain or loss will be the same with different toxic substances, nor is this the case.

4. SULPHURIC ACID, OSMIC ACID AND MERCURIC CHLORIDE

These substances influence the swelling of potato rather similarly to those just discussed, that is, there is a preliminary period of
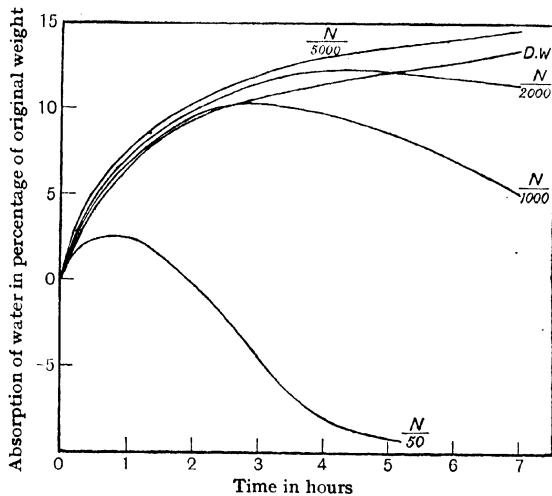
Fig. 12. Curves illustrating the influence of sulphuric acid on the absorption of water by potato tuber. (After Stiles and Jørgensen.)

swelling followed by a period of shrinkage. There is no doubt that acids are absorbed by plant tissue, the evidence for which will be discussed in a subsequent chapter. The results obtained for sulphuric acid are shown in Fig. 12. It will be observed that in a concentration of $N/50$ the intake of water reaches a maximum in about an hour, while in a concentration as low as $N/2000$ the swelling gives place to shrinkage after 4 or 5 hours. Even in $N/5000$ sulphuric acid shrinkage supervened before the lapse of 24 hours while in distilled water the tissue was still undergoing slow swelling after this lapse of time.

A curious phenomenon noted in the case of sulphuric acid is that in the lower concentrations the tissue actually absorbed water at more rapid a rate at first than in distilled water. Calculation of the probable error of experiment left no doubt that this is not to be accounted for on account of the variability of different samples of tissue. This phenomenon is rather difficult to account for, since as the concentration of acid external to the tissue is so low, the penetration of it into the cell sap could scarcely increase the osmotic pressure of the latter sufficiently to account for the increased rate of water absorption. It is possible that this increased swelling may be referred to the increased absorption of water by proteins and other colloidal systems in acid solutions as compared with distilled water, but definite evidence confirmatory of such a view is wanting.

Thoday (1918 a) has shown that a similar increase of rate of water absorption in lower concentrations of the solute as compared with that in distilled water is to be observed in the case of osmic acid and mercuric chloride. It may be significant that mercuric chloride undergoes partial hydrolysis in solution so that the solution contains a small quantity of hydrochloric acid.

5. ETHYLIC ACETATE AND PHENOLS

The absorption of ethylic acetate by grains of barley was examined by Brown and Worley (1912). Ethylic acetate enters the grain, but the rate of water intake is more rapid than from pure water. This was regarded by Brown and Worley at the time as possibly due to a simplification of the water molecule in presence of this ester. Later Brown and Tinker (1916 a) observed a similar phenomenon in the case of various phenolic solutions. Barley grains absorbed various solutions of phenol of different concentrations the more rapidly the more concentrated the solution. With a number of different phenols in half-normal concentration the rate of absorption was in this order: phenol, catechol, resorcinol, pyrogallol. The last entered at the same rate as water. Since variations in osmotic pressure, vapour pressure and viscosity of these various solutions are slight, while the rate of absorption is in inverse order to the surface tensions of the solutions (Worley, 1914), Brown and Worley conclude that surface tension is also a factor in determining the rate of entrance of solutions into barley grains.

CHAPTER XI

THE DETERMINATION OF THE PERMEABILITY
OF PLANT CELLS TO DISSOLVED SUBSTANCES

THE passage of dissolved substances into living cells takes place for the most part independently of the absorption of water. Just as the latter enters the cell in order to bring about equilibrium on the two sides of the cell membranes, so the passage of a dissolved substance into the cell can only proceed until the distribution of the substance within and without the cell fulfils the conditions of equilibrium. Of course, if the passage of water into, or out from, the cell should alter the conditions of equilibrium with regard to dissolved substances, passage of dissolved substance across the cell membranes may take place, but there appears no reason for supposing there is any closer connexion between the entrance of water and solutes into plant cells.

The entrance of any solute into a cell depends therefore not only on the permeability of the cell membranes to the substance, but also on how far the distribution of the substance on the two sides of the membrane is removed from the equilibrium conditions. For this reason, although the passage of a substance into the cell can be taken as evidence of the permeability of the cell to the substance, the non-entrance of it need not necessarily indicate that the cell is impermeable to the substance. Similarly the rate at which a substance enters a cell cannot be taken as a measure of the permeability of the cell. If a dissolved substance were able to penetrate the protoplasm and combine with some constituent of the cell, it would obviously enter the cell to a much greater extent and at a greater rate, than if it did not combine with any constituent. In the latter case it might

even be difficult to discover whether the substance were permeable
at all to living cells. For many substances which are harmless to
cells when presented to them in low concentration have a toxic effect
in stronger concentrations, and as a result of toxic action the per-
meability of the cell may be greatly increased. It may therefore
often be necessary to use substances in dilute solutions if permeability
of *living* cells is to be examined, and if no marked accumulation of the
substance in the cell sap takes place its appearance there may be very
difficult to recognise on account of the small quantity of it present.
The necessity of distinguishing between intake or absorption of a
substance by the cell and the permeability of the cell membranes
to the substance has been rightly emphasized by recent writers
(Höber and Nast, 1913; Brooks, 1917 *a*; Osterhout, 1917 *d*).

It may again be emphasized in this place that the cell is a very
complex system with regard to its permeability. A dissolved sub-
stance in diffusing into the cell sap from the solution outside the cell
has to pass through the cell wall and the protoplasm and possibly
through plasmatic membranes on the inner and outer surfaces of the
protoplasm which differ in permeability properties from the bulk of
the protoplasm (cf. Stiles and Jørgensen, 1918). It is very generally
impossible to distinguish between these different phases in attempt-
ing an analysis of permeability phenomena, so that in the following
where the permeability of the cell or of the cell membranes is men-
tioned it means the permeability of the whole series of cell membranes
which separate a liquid external to the cell from the cell sap in the
vacuole.

It should be perfectly clear from what has been said of the per-
meability of membranes in Chapter V, that we may expect the per-
meability of the same cell to differ exceedingly towards different
solutes. We may also expect that the order of permeability to a
series of substances may differ in the case of different cells. Also as
cells differ so much in their structure and properties we may expect
that methods applicable for the determination of the permeability
of some kinds of cells may not be applicable in the case of others.
This is indeed the case, as will be very evident from a consideration
of the various methods that have been evolved for the determination
of permeability. These methods are described below.

● 1. DIRECT TEST OF PERMEABILITY BY OBSERVATION OF VISIBLE CHANGES IN THE CELL

If a substance on entering the cell should produce a visible change in it, it is obvious that such visible change is evidence of the permeability of the cell to the substance. '

(i) *Dyes.* The simplest application of this method is the investigation of the penetration of dyes into the cell, the permeability of which to a dye is shown by the coloration of the cell contents by the penetrating dye. Using this method Pfeffer (1886) examined the permeability of living cells of a considerable number of plant species to a number of dyes. He found that a passage of the dye into living cells took place with methylene blue[1], methyl violet, cyanin, Bismarck brown, fuchsin, safranin, methyl orange, tropæolin ooo, methyl green, iodine green, Hoffmann's violet, gentian violet and rosolic acid. These substances are taken up by the cell from very dilute solutions and accumulate in the cell sap so that the concentration inside the cell must be much higher than that of the external solution. Thus methylene blue diffuses into the root hairs of *Trianea bogotensis* and the outer cells of the root of *Lemna minor* among others, so as to give a deep blue colour to the cell sap even when the solution of the dye presented to the cells is as dilute as 0·0008 per cent. In some cases some of the dye does not accumulate in the vacuole in solution, but in the form of a blue precipitate as in the root hairs of *Azolla caroliniana* and in *Spirogyra communis*. When this is the case, continued immersion in the dye may lead to accumulation of the dye in solution in the cell sap in addition to the precipitate, as observed by Pfeffer in *Zygnema cruciatum*. The blue precipitate appears to be a compound of the dye with tannin, and accumulation of the dye in solution in the vacuole apparently only takes place when all the tannin present has combined with the dye. Pfeffer supposes the accumulation of the dye in solution in the vacuole is due to the formation of an undetermined soluble compound of methylene blue with some substance or substances present in the cell sap, and that the cell mem-

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[1] In the English edition of Pfeffer's *Physiology of Plants*, 1 (1900), pp. 94, 96, 97, where these observations of Pfeffer are summarised, this dye is most unfortunately referred to as methyl blue, a dye which Pfeffer showed was incapable of penetrating into the cells he examined. In Pfeffer's original paper an unfortunate misprint occurs, methylene blue being included in the list of dyes which penetrate plant cells and also in the list of those which do not. The body of the paper makes it quite clear that in the latter case "methylen-blau" is a misprint for "methylblau."

branes are impermeable to the substance produced. There may, of course, be a number of different substances present in the cell capable of forming non-diffusible compounds with methylene blue of other dyes, but the only substance in addition to tannin which has so far been recognised as capable of this appears to be phloroglucinol (Waage, 1890; Klemm, 1892). It might also be possible to account for the accumulation of the dye in the cell sap by adsorption. If this were so, one would expect the highly colloidal protoplasm to play a greater part in adsorbing the dye than the less highly colloidal cell sap. Nevertheless in the case of methylene blue no appreciable coloration of the protoplasm by the dye is observable, but on the other hand, rosolic acid was observed to enter the protoplasm so that the latter became visibly coloured, whereas no accumulation of the dye in the vacuole was observed. All the other dyes recorded above as entering the cell both accumulate in the vacuole and stain the protoplasm. How such "vital staining," that is, the staining of living cells by the accumulation of dye ("intra-vitam stain") in the cell, takes place, will be considered in a later chapter.

With nigrosin, aniline blue, methyl blue, marine blue, aniline grey, eosin and congo-red, no visible intake of the dye into living cells could be observed, although penetration may be rapid enough into dead cells. Even after three days' immersion in a 1 per cent. solution of aniline blue or a 0·5 per cent. solution of nigrosin no coloration was observable of the cell sap of (?) root hairs of *Trianea bogotensis* immersed in these very densely coloured solutions, and the cells were uninjured. Similarly indigo-carmine when presented to *Spirogyra setiformis* and *Trianea* in 0·7 per cent. solution, was not absorbed even after four days; even the cell walls were not stained and the cells remained alive.

Apparently none of the dyes examined by Pfeffer are absorbed either by the nucleus or chromatophores if the cell remains alive. Only after the death of the cell can the nucleus become stained, and methylene blue, for example, can then be absorbed by the nucleus. But Campbell (1888) working in Pfeffer's laboratory showed a little later that the nucleus of living cells can be stained by some dyes, as for example, dahlia and mauvein. Other cases have since been recorded by Lauterborn (1893) and Palla (1893), for example. This possibility was fully recognised by Pfeffer, who pointed out that indigo-carmine had been shown to stain the nuclei of living kidney cells (Heidenhain, 1874), while different kinds of cells may vary in regard to the staining of bodies contained in them. Thus the micro-

somes in *Trianca* are usually intensively stained, while those of *Sapro-legnia* and *Momordica* remain uncoloured.

This method has been much used to obtain information in regard to the mechanism of absorption and permeability, as, for example, by Overton (1899, 1900) who concluded that those dyes are absorbed by the cell which are soluble in lipoid substances, and Ruhland (1908 *a, b,* 1909 *a*) who at one time held that the uptake or non-uptake of a dye by living cells depends on whether the dye is basic or acid[1] respectively. This view was combated by Höber (1909) who had shown that a number of acid dyes were capable of staining the epithelial cells of the kidney. Ruhland supposed that the cell might be permeable to the acid dyes but that they combined with no cell constituent and so were not accumulated in the cell and consequently did not stain it sufficiently for the dye to be visible. The recent work of Collander (1921) indicates however that certain sulphonic acid dyes examined by him do not enter a variety of plant cells to any appreciable extent. Thus most cells placed in solutions of cyanol extra, orange G, methyl orange, Ponceau R and wool violet S for 24 hours or more became coloured with the dye to only $\frac{1}{8}$ to $\frac{1}{100}$ of the depth of colour of the external solution, while analysis of the external solution showed that this was the true explanation and that the dye was not absorbed and so not accumulated in the cell as a colourless compound of dye with a cell constituent.

Certain cells, however, were shown by Collander to be capable of accumulating these sulphonic acid dyes. Such are the cells surrounding the vascular bundles of the perianth leaves of a white flowering variety of *Hyacinthus orientalis*, and similar cells in a white flowered variety of *Tulipa Gesneriana*, a fact discovered by Rohde (1917).

A variant of the method was introduced by Küster (1911), who investigated the absorption of dyes from the cut surfaces of shoots or other organs immersed in a solution of dye. He found that a number of acid dyes were capable of passing into and staining cells when introduced to the cells in this manner. This conclusion was confirmed by Ruhland (1912 *a, b*) who also found that out of 30 basic dyes tested several were not absorbed by the bulb scales of the onion, namely, Victoria blue 4R and B, Basler blue R and BB, gallamin

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[1] A basic dye is one in which the radicle to which the colour is due is a base. Thus Bismarck brown is the colour base triamidoazobenzene

$$NH_2C_6H_4N:NC_6H_3(NH_2)_2;$$
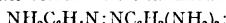
it is generally used as the hydrochloride. Similarly, in an acid dye the radicle to which the colour is due is an acid. Congo-red is such a case, in which the dye as used is generally the sodium salt of the acid.

blue and night blue, while two were absorbed only slowly, namely, diazin green and Victoria blue R.

The significance of these results in regard to the mechanism of cell permeability will be dealt with in a later chapter.

This method is obviously chiefly useful as affording a qualitative test of the penetration of dyes into plant cells, and although it furnishes indisputable evidence of the entrance of a penetrating dye, it is not safe to use it as evidence of the non-penetration of a dye when the cell sap remains uncoloured. Nor is the method a very suitable one for quantitative measurements. Nevertheless, Collander has made it so by mounting sections of tissue immersed for a certain time in the dye in solutions of the dye of different concentrations and so comparing the colour of the section with that of the surrounding solution. The solution the colour of which appeared of the same depth as that of the section is taken as having the same concentration as the dye in the cell sap. In this way Collander showed that after immersion in quite strong solutions of sulphonic acid dyes, the concentration of the cell sap in regard to dye is usually only a fraction of that of the external solution.

(ii) *Acids and alkalies.* If the cell should contain a substance which acts as an indicator for acid or alkali, the passage of substances belonging to one or other of these groups can be detected by the change in colour of the sap. De Vries (1871 *a, b*) observed the permeability of the cells of the root of red beet to ammonia by the change in colour of the pigment in the cells from red to blue.

Pfeffer (1877) extended this observation to other plant cells with coloured sap, such as the petals of flowers of *Pulmonaria* and the staminal hairs of *Tradescantia*, and showed that such cells are readily permeable to acids, including weak acids such as carbonic, acetic, tartaric and phosphoric acids, and to alkaline hydroxides as well as ammonia. If the reagent is used in dilute enough solution and the tissue well washed with water soon after treatment so that the acid or alkali is quickly removed, the original colour of the sap may return and the cell apparently suffer no injury by the treatment.

Haas (1916 *a*) found a number of tissues suitable for examining the permeability of acids and alkalies by this method, these tissues including the petals of *Browallia speciosa*, *Pelargonium* and *Hyacinthus orientalis*, var. *Queen of the Blues*, and the root of red radish. The rate of penetration of a number of acids and alkalies through these tissues was compared, the substances being used (1) in a concentration of 0·01 N, and (2) in such a concentration that the hydrogen-

ion or hydroxyl-ion concentration was 0·01 N. In the former series and with *Browallia* petals the order of alkalies was ammonium hydroxide, sodium hydroxide and potassium hydroxide, while the order of acids was salicylic, benzoic, trichloracetic, formic, hydrochloric, nitric, sulphuric, phosphoric, oxalic, tartaric (lactic, citric), acetic. The acids included in a bracket penetrated at the same very slow rate while the rate of penetration of acetic acid was extremely slow. The order of acids in the case of *Hyacinthus* perianth was much, but not quite, the same. In the second series, on the other hand, in which the acids were used with the same hydrogen-ion concentration, the order of penetration was quite different, acetic acid penetrating most rapidly of all the acids examined.

More recently Brenner (1918) has recorded observations made by this method, the tissue used being principally hypodermal cells of red cabbage and staminal hairs of *Zebrina pendula*. His conclusions are not identical with those of Pfeffer. Various acids, namely, hydrochloric, nitric, sulphuric, phosphoric, citric, malic, tartaric and oxalic acids, are stated to penetrate the undamaged protoplasm very slowly if they are presented in low non-toxic concentrations, while the somewhat higher concentrations, from which the acids enter more rapidly, injure the cell, which apparently accounts for the greater ease of penetration. The vitality of the cell after such treatment can be tested by plasmolysis and deplasmolysis, and, if the cells are suitable, by protoplasmic movement.

Bethe (1909), Warburg (1910) and Harvey (1911) have extended the method to colourless cells by first allowing the intra-vitam stain neutral red to penetrate the cells, which are thus stained red. Neutral red is an indicator, changing colour to yellow in alkaline medium. By this means Harvey showed the rapid penetration of ammonia and amines, whereas the strong bases diffused much less readily into living cells, and even their entrance might have been due to their toxic action rendering the cells more permeable.

(iii) *Salts other than dyes.* If a cell contains a substance with which a salt reacts to give either a precipitate or a different colour, the penetration of the salt into the cell can be observed by means of the formation of the precipitate or colour. Thus C. Darwin (1875) showed the penetration of ammonium carbonate into the root of *Euphorbia peplus* by the cloudy appearance produced in the cells. The aggregation produced by the same salt as well as by ammonium acetate and a number of other nitrogenous substances in the tentacles of *Drosera* was attributed by Darwin to the penetration of the salt or other

dissolved substance into the cells. Again if a cell contains soluble calcium salts, the entrance of a soluble carbonate or oxalate will result in the formation of a precipitate of the corresponding calcium salt. In this manner Pfeffer was able to show the permeability of cells to ammonium carbonate. Conversely if a cell contains soluble oxalates or carbonates, the permeability of the cell to calcium salts can be similarly determined. Osterhout (1910) showed that root hairs of very young seedlings of *Dianthus barbatus* formed crystals of calcium oxalate when the roots were in a solution of a calcium salt, but not otherwise. The following solutions were used: 0·005 M calcium sulphate, 0·005 M calcium nitrate, 0·005 M calcium chloride, 0·0001 M calcium hydrate, dilute artificial sea water and tap water. Crystals of calcium oxalate made their appearance in root hairs of *Dianthus barbatus* within four hours at 30° C. after immersion in the solutions, and in the case of calcium sulphate in 1·5 hours.

The penetration of iron salts into cells rich in tannin is rendered visible in the cell by the production of a blue colour. By the use of this test Miss Williams (1918 b) was able to show that hypodermal cells of the leaf stalks of London pride (*Saxifraga umbrosa*) are normally impermeable to ferric chloride, but that after treatment with various electrolytes the protoplasm becomes permeable to ferric chloride which enters the cells at such a rate as to give a definite blue colour within three minutes, whereas before treatment such a coloration is not produced after immersion for two hours in this salt.

The absorption of copper salts by a tree of *Quercus macrocarpa* was recognised on account of the metallic copper which accumulated in the cells (cf. MacDougal, 1899). Accumulation is made possible in this case by the reduction of the copper in the penetrating salt to metallic copper, so that equilibrium between the copper salt inside and outside the cell is not reached and endosmosis of the salt therefore continues.

(iv) *Sugars and Glycerol.* The penetration of glucose, sucrose and glycerol into the cells of water plants such as *Lemna*, *Elodea* and *Potamogeton*, can be demonstrated by the formation of starch from the absorbed sugars or glycerol inside the cells (Boehm, 1883; Meyer, 1886; Acton, 1890). The absorption of the sugar or glycerol takes place in the dark, while in control experiments with the same plants not provided with sugar or glycerol, no starch is formed.

Ruhland (1911) used this method to examine the permeability of the cells of sugar beet leaves to a variety of sugars. He found that raffinose, sucrose, maltose, glucose, fructose, certainly entered the

cells, as starch was formed in considerable quantities when the leaves were provided with these sugars in the dark. Galactose, mannose, sorbose, rhamnose and glycerol also entered the cells as indicated by the formation of starch, but no starch was formed in leaves provided with arabinose, xylose, erythrite, mannitol or dulcitol. However, this is not evidence of non-penetration of these substances, as the leaf cells may not be able to utilise them in the production of starch.

The value of similar evidence for the penetration of formaldehyde is slight (Spoehr, 1916; Jørgensen and Stiles, 1917).

(v) *Other substances.* A number of substances not included among the classes already mentioned will combine with some constituent of the cell sap to produce insoluble substances which thus become visible in the cell as precipitates. The penetration of the purine caffeine into cells was noted in this way by Pfeffer, the caffeine producing a precipitate with tannin contained in the cell. The permeability of antipyrin was observed in the same way by Pfeffer.

Similarly the penetration of substances producing colour in the cell can be observed. Thus Miss Williams (1918 *a*) has shown that living mycelia of *Penicillium glaucum* and *Oidium lactis* can absorb gold from colloidal solution, but that the gold is absorbed and retained by the cell walls which are stained blue.

2. DETERMINATION OF PERMEABILITY BY MEANS OF MICROCHEMICAL TESTS

The entrance of many substances into the cell can be made evident by treating the cell afterwards with some microchemical reagent which can be used as a test for the particular substance under examination. By this method Janse (1887 *b*) investigated the permeability of a number of plant cells to potassium nitrate. Following this substance the cells were treated with diphenylamine which gives a blue colour with nitrates (Molisch, 1883). Cells of *Chætomorpha aurea, Spirogyra nitida, S. crassa, S. communis, Tradescantia discolor, Curcuma rubricaulis* and *Stratiotes* were all found to be permeable to the inward passage of potassium nitrate. Wieler (1887) performed similar tests with seedlings of *Phaseolus multiflorus, Vicia Faba* and *Helianthus annuus*, testing in addition for potassium in the plants with platinic chloride with positive results. This method is obviously capable of wide extension as a qualitative test of permeability and absorption. It does not lend itself in its present form to quantitative measurements of either,

3. DETERMINATION OF PERMEABILITY BY ANALYSIS
OF THE EXPRESSED SAP OF CELLS AND TISSUES
OR OF CELL OR TISSUE EXTRACTS

Investigations of cell permeability by analysing the expressed sap of the plant material used after immersion in the experimental liquid have been made by a number of authors. The method is generally not applicable to single cells as only rarely can enough liquid be obtained from a single cell for the purposes of analysis, and in the case of cells forming part of tissues the individual cells cannot, of course, be separated from one another. Nevertheless, in two cases at least the method has been applied to single cells. Wodehouse (1917) made determinations of the permeability of single cells of the marine alga *Valonia* by this method. The "cells[1]" of this plant are so large that 1 to 2 c.c. of sap can be pressed out from cells of normal size, and up to 5 c.c. from large cells. Qualitative examination showed that potassium was much more abundant in the vacuole than in the surrounding medium (sea water), sodium and calcium were both present, but not more than a trace of magnesium although this occurs in some quantity in sea water. Chloride was present in high concentration in the cell sap, but sulphate was absent although it occurs in fairly high concentration in sea water. Conversely nitrate was found in the vacuole, whereas in sea water there was not enough to be detected by the qualitative tests employed[2]. The cell wall appears to play no part in the selective absorption thus demonstrated, for if living cells are killed and then replaced in sea water sulphates are soon present inside the cell.

The same method has recently been advocated for testing the penetration of dyes into living cells of *Nitella* (Irwin, 1922 *a, b*). The cells are so large that sufficient sap can be pressed out from a single cell for a colorimetric determination of the concentration of the dye that has penetrated into the cell. In this way it is held that quantitative investigation on the permeability of these cells to dyes is possible.

A method employed by Janse (1887 *b*) for testing the permeability of *Spirogyra* cells to potassium nitrate consists essentially in testing an extract of the cells with diphenylamine. Filaments of the alga, after immersion for various times in potassium nitrate solution, were made to burst in water containing diphenylamine. If the time of immersion of the *Spirogyra* in potassium nitrate solution had been

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[1] *Valonia* is a cœnocyte and the compartments are not regarded as true cells.
[2] These results have recently been confirmed by quantitative analysis (Osterhout, 1922 *a*).

sufficiently long a blue coloration was produced indicative of the fact that nitrate had diffused into the cells previously. The result of the test was negative if the time of immersion in the nitrate was short. '

The analysis of tissues, expressed sap and extracts of tissues has been used considerably as a test of permeability. Thus Nathansohn (1903) made analyses of the expressed sap of the marine alga *Codium* after immersion in a solution of sodium nitrate and was in this way able to obtain data with regard to the penetration of sodium and ' nitrate ions into this plant. Paine (1911) used the same method to investigate the permeability of yeast cells to inorganic salts, and concluded that the slight intake he observed was entirely due to adsorption of the salt by the cell wall.

Analyses of whole plants growing in soil, water culture and other media have afforded much information with regard to the relative intake of different substances by living normal plants. The long series of ash analyses of various plants made by Wolff (1864–1880) and others has shown that compounds of potassium, sodium, calcium, magnesium and iron, and compounds containing chlorine, sulphur, phosphorus and silicon are present in considerable quantity in practically all plants examined, and it must be therefore concluded that such compounds are capable of penetrating into the cells of plants. Aluminium and manganese also appear to be regular constituents of plant ash, though often in very small amount, so it must be supposed that compounds containing these substances are capable of penetrating through the cell membranes of plants. The relative quantity of these various ash constituents varies however in different species (cf. for example, Grandeau and Bouton, 1877), and the differences cannot be correlated with differences in composition of the medium from which the plant obtains its ash constituents, although plants of the same species can take in more of any particular constituent from a medium which is rich in the constituent than, from a medium poor in it (cf. Malaguti and Durocher, 1858, for the case of calcium).

Apart from the work of Salm-Horstmar (1851, 1856) with soil cultures and Sachs (1860, 1861) and Knop (1860) with water cultures, directed to determine the elements essential for plant nutrition, the work of Mazé (1913 *a, b*, 1914), Monnier (1905), Déléano (1907, 1908 *a, b*) and Pouget and Chouchak (1913) deserves mention in this place, as these authors have used the method of chemical analysis to determine the intake of different mineral constituents at different stages in the development of the plant. Their results were obtained, however, from the point of view of the part played by inorganic salts

in the development of the plant, and have only a passing interest from the point of view of permeability.

• The chemical analysis of the whole plant can, of course, also be employed as a test of the permeability of the cells to substances other than salts. Thus Bourget (1899) showed that members of the Liliaceæ and Chenopodiaceæ were able to absorb considerable quantities of iodine through their roots, while the cells of the roots of potato were apparently impermeable to this element. Similarly, it is well known that some marine algæ absorb iodine from sea water to such an extent that they have been used as a commercial source of that element. By direct analysis Stoklasa, Šebor, Tymich and Cwacha (1922) have shown that aluminium ions are absorbed by the hydrophytes *Galeopsis versicolor* and *Caltha palustris* and the mesophytes *Dactylis glomerata* and *Festuca pratensis*, but scarcely by the xerophytes *Sesleria cærulea* and *Anthyllis Vulneraria*.

Brooks (1917 *a*) raises the objection to this method that the quantity of substance found by analysis of tissue extracts includes any of the substance held in the cell walls and intercellular spaces. It is clear that the method cannot be used as a method for the quantitative measurement of *permeability* of the cell membranes, as other factors, namely, adsorption and chemical action, may influence the quantity and rate of absorption of the substance. When expressed sap is analysed there is the additional objection that it is difficult to obtain a reliable sample of sap, although this difficulty can apparently be overcome by suitable methods of extraction (cf. Chapter IX).

4. DETERMINATION OF PERMEABILITY BY VISIBLE CHANGES IN THE EXTERNAL MEDIUM

Visible changes in the external solution can be used in some cases as a test of the permeability of the cell membranes to substances contained in the cell. Thus when the cell sap contains a pigment the diffusion of this out of the cell can be observed by the coloration of the external liquid as soon as sufficient of the pigment has exuded, and the quantity that has passed out from the cells or tissues employed can be estimated colorimetrically (cf. Stiles and Jørgensen, 1917 *a*). If the external solution should contain an indicator, the diffusion out of acids or alkalies can similarly be observed, while if any of the constituents of the cell sap should give a precipitate or a colour reaction with a substance present in the external liquid, the exosmosis of this constituent can be similarly observed. The disad-

vantage of this method of studying exosmosis is that the quantities of diffusing substances may be too small for determination.

The change in colour of a solution of a dye external to the cells or tissue can similarly be used to determine the absorption of the dye, and by colorimetric estimation the method may be made quantitative. The chief objection that can be levelled against this means of determining intake is that a substance diffusing out from the tissue might react with the dye to produce a colourless or differently coloured substance, so that a decrease in the intensity of the colour of the external solution might not be due actually to an absorption of the dye by the tissue. That this may happen in some cases appears to be suggested by an observation of Miss Redfern (1922 *b*) to the effect that a solution of congo-red *deepens* in colour after disks of carrot root are immersed in it for some time. As such a deepening of colour does not occur in a solution of the dye to which no tissue has been added, the only possible explanation appears to be that the colour is altered by the exosmosis of some substance from the tissue. A more serious difficulty arises from the fact that the dye may be absorbed by the cell walls and never reach the protoplasm or vacuole. While this appears to be the case sometimes, it is not always so, and clearly observations by this method are free from objection if supported by others following the first method described in this chapter.

5. Determination of Permeability by Chemical Analysis of the Medium External to the Tissue

This method is similar to the one previously described, but as a quantitative method of determining absorption it is as a rule more refined. It has found considerable use as a method of investigation of cell permeability and intake or excretion. A decrease in the concentration of a substance in the external solution indicates absorption of the substance by the cells or tissue while increase in its concentration indicates exosmosis. As a qualitative test of permeability and a quantitative measurement of absorption and excretion the method is open to the same objections as that just described. It cannot be used quantitatively for measuring permeability any more than other methods described previously in this chapter.

Information relating to absorption of salts has been obtained by this method by a number of workers, both entire plants and pieces of tissue having been used. Among those who have worked with the former may be mentioned particularly Demoussy (1900) who deter-

mined the relative absorption of potassium and calcium by wheat
and maize, Colin and de Rufz de Lavison (1910 *a, b*) who investigated
the absorption of barium, strontium and calcium, Pantanelli (1915 *a,
b, c*, 1918) and Pantanelli and Sella (1909) who paid particular atten-
tion to the unequal absorption by a number of species of the two ions
of the same salt, and Miss Redfern (1922 *a*) who investigated the same
problem critically in the case of the absorption of calcium chloride
by the edible pea and maize. The absorption of glucose by the roots
of the latter plant was shown by Laurent (1897).

Absorption of salts by disks of storage tissue was examined by
Nathansohn (1903, 1904 *a, b*), Meurer (1909) and Ruhland (1909 *b*).
The results obtained by these various workers will be dealt with in
the next chapter.

The exosmosis of any particular substance can, of course, be
examined by this method. Thus it has been shown that sugars, chiefly
sucrose, can diffuse out of the cells of the leaf of the sugar beet when
the leaf is immersed in water (Puriewitsch, 1898; Ruhland, 1911).

6. Determination of Permeability by Measurement of the Electrical Conductivity of the External Solution

The exosmosis of electrolytes from plant tissue into a surrounding
medium consisting of pure water or a solution of a non-electrolyte
will result in an increase in the electrical conductivity of the external
solution. Measurement of the electrical conductivity of the latter can
therefore be used as a criterion of the permeability of the cell mem-
branes to the electrolytes of the cell. The method cannot of course
be used to determine the exosmosis of any particular electrolyte,
but it can be used for approximate quantitative determinations
of exosmosis by assuming the electrical conductivity of the ex-
ternal solution to be a measure of the concentration of electrolytes
in the solution. This is, of course, only approximately true, since the
mobilities of different ions are different and with increasing concen-
tration of the electrolytes the degree of dissociation will decrease,
and the electrical conductivity is dependent on the mobilities of the
ions and the degree of dissociation. However, with the exception
of hydrogen and hydroxyl ions, the mobilities of ions likely to diffuse
out of plant cells do not differ greatly among themselves, while the
degree of dissociation will not be depressed greatly in the dilute
solutions likely to be involved. If this external solution contains a
non-electrolyte the presence of this will depress the conductivity,

and in comparative experiments allowance may have to be made for this (Stiles and Jørgensen, 1917 *a*).

Using this method Briggs and True and True and Bartlett (1912) showed the excretion of salts into distilled water by the roots of seedlings of *Lupinus albus* and field peas, while Stiles and Jørgensen (1917 *a*) investigated the influence of a number of organic substances on the exosmosis of electrolytes from potato tuber tissue.

The method is also usable within limits to determine the intake of electrolytes by plant tissue. Here a complication arises because intake of the salt by the tissue and excretion of electrolytes by the tissue will influence the conductivity in different directions. Interaction of the exudate and the constituents of the external solution may also be a disturbing factor. Results obtained may thus be sometimes very difficult to interpret (Stiles and Jørgensen, 1915 *a*). Nevertheless, by allowing for the exosmosis it may be possible to obtain quantitative results as has been done in regard to the absorption of a number of salts by carrot root by Stiles and Kidd (1919 *a, b*), who have pointed out the causes making for a fall or rise in the conductivity in the external solution, and have shown that in the results published by them the values obtained can be regarded as minimum values for absorption. These results will also be dealt with in the following chapter.

7. DETERMINATION OF PERMEABILITY AND ABSORPTION BY
MEANS OF ELECTROMETRIC MEASUREMENTS WITH THE
HYDROGEN ELECTRODE

The exosmosis of acids and alkalies can be determined by measuring the concentration of the hydrogen ions in the external solution by means of the hydrogen electrode. A description of the method and the various forms of electrodes that have been devised will not be entered into here; those interested should consult the recent work of Clark (1920) in which the measurement of hydrogen ions is adequately treated. Similarly the absorption of hydrogen or hydroxyl ions from an acid or alkaline solution can be determined. The method has been used for measuring the absorption of hydrogen ions from dilute solutions of hydrochloric acid by Stiles and Jørgensen (1915 *b*), and of a number of other acids by Miss Hind (1916).

8. Determination of Permeability and Absorption by Colori-
metric Estimation of the Hydrogen Ion Concentration
OF THE EXTERNAL SOLUTION

Changes in the concentration of hydrogen ions in the external
solution can also be determined colorimetrically by the indicator
method (see Clark, 1920). The scope of the method is exactly the
same as the preceding, although the results obtainable are not so
accurate. Using this method Haas (1916 b) has shown that no excre-
tion of acid apart from carbonic acid takes place from the roots of
maize and wheat dipping into distilled water. No excretion of alkali
occurs in the case of maize, while with wheat there is only a very
slight increase in the alkalinity of the water after the roots have
decayed.   .

9. Test of Permeability by Metabolic Action

While ash analyses have shown what substances are normally
absorbed by plants, growing plants on artificial media of known com-
position has shown which elements are necessary for normal growth.
If such an element is supplied to a plant in only one compound and
the plant grows healthily so that its metabolism is normal, it follows
that that substance[1] must be able to penetrate into the plant. Thus
it has been shown that although nitrogen is most suitably provided
to most higher plants in the form of nitrates, in many cases other
nitrogen compounds are readily absorbed. Thus ammonium salts,
urea, various amino-acids such as glycine, asparagine, leucine and
tyrosine, uric and hippuric acids, acetamide and propylamine are
among nitrogen compounds that have been shown to be absorbed by
various species by means of the continued growth of the plants when
supplied with no other source of nitrogen. The literature dealing with
the possible sources of nitrogen for Fungi and Bacteria is enormous
and cannot be entered into here.

The penetration of inorganic salts through the leaves of *Thunbergia*
and other plants was shown in this way by supplying the roots with
nothing but water and spraying the leaves with a solution of nutrient
salts, or by immersing them in the solution (Dandeno, 1901).

The healthy growth of algæ such as *Nostoc* (Bouilhac, 1897) and
Stichococcus (Artari, 1901; Matruchot and Molliard, 1902) in the dark
on solutions containing glucose or other organic sources of carbon
indicates the permeability of the cell membranes to the sugar or
other organic nutrient.

[1] Or, in the case of an electrolyte, one ion of it.

In a similar way the unhealthy development or the death of a plant may be taken as evidence of the penetration into it of a poisonous substance if this is present in the external medium in addition to all the necessary nutrients. The test is equally legitimate if the presence of a dispensable substance in the external medium should have a stimulating effect on the plant and bring about a development above the normal. Thus the penetration of copper salts into roots of cereals and other plants has been made abundantly clear from the work of Phillips (1821) and many later writers, particularly Otto (1893), Coupin (1898), Kahlenberg and True (1896), Copeland and Kahlenberg (1900), True and Gies (1903) and Miss Brenchley (1910) among others. In a similar way the absorption of compounds of zinc, arsenic, manganese and boron has been shown, for a review of the work on which the reader is referred to the paper and book of Miss Brenchley (1914 *a, b*) dealing with the effect of these substances on plant growth. In Miss Brenchley's book will be found abundant references to the literature of the subject. Even compounds of the alkali metals (Coupin, 1901) have been shown to penetrate the roots of cereals by their harmful effect on vegetative development. The penetration of cyanides has been indicated in the same way (Brenchley, 1917).

Since this method can only afford a qualitative test of permeability, and has done so only incidentally, it is not worth while to deal with it in any greater detail here. It must, however, be pointed out, that the toxicity of a substance may indeed be a clear indication that it *reaches* the protoplasm, but it is no indication that the substance has the power of *passing through* the protoplasm before the properties of the latter, in respect of permeability, have been completely changed by the action of the substance. The test of toxicity is perhaps the least useful of all the tests of permeability.

10. PLASMOLYTIC METHODS

Concentration required to produce plasmolysis. It has already been pointed out in Chapter IX that a solution of a substance, incapable of penetrating into the cell, which just does (or just does not) produce plasmolysis, is, on the simple osmotic view of the cell, isotonic with the cell sap at the moment when plasmolysis is just about to commence. It must be pointed out, however, that if the sustance penetrates the cell sap but is immediately withdrawn from solution to form an insoluble compound, plasmolysis may still occur if the quantity of salt withdrawn from the external solution is insufficient to alter materially the concentration of the external solution. It is not

even necessary that an insoluble compound should be formed so long as the new combination of substances in the vacuole does not exert any higher osmotic pressure than previously. That such possibilities are by no means to be ignored is made very clear from the work of Pfeffer on the penetration of dyes described earlier in this chapter. Similarly, Stiles and Jørgensen (1917 *b*) have shown that storage tissues actually plasmolyse in solutions of common salt and remain so for 20 hours or more, while Stiles and Kidd (1919 *a*, *b*) have shown that sodium chloride is actually absorbed with some rapidity by such tissues.

But if such complication does not enter into the case, the penetration of a substance into the cell will raise the osmotic concentration of the cell sap, and hence the external solution will no longer be able to effect plasmolysis. In order to bring this about a higher concentration of the solution will be necessary, and even then, if the entrance of solute continues, the cell will recover from plasmolysis.

If then the osmotic concentration of a substance required to produce plasmolysis is higher than that required in the case of a substance which is known not to penetrate the cell, it may be concluded that the former substance penetrates the cell.

Sucrose is generally chosen as a substance which penetrates the cell extremely slowly or not at all, and it is fortunate that the values of the osmotic pressure of this substance are more firmly established than those of other substances. The concentrations of other substances isotonic with sucrose solutions have been determined chiefly by indirect methods, such as calculations from the freezing point lowering, or from determinations of the electrical conductivity, as very few direct determinations of osmotic pressure of substances other than sucrose have been made (cf. Chapter VI). If the isotonic coefficient found for a substance by the plasmolytic method is lower than that found by direct measurement or by calculation from other physico-chemical data, it is concluded that the cells used for the determination of the coefficient are permeable to the substance examined.

In this way de Vries (1888 *a*, *b*, *c*) showed that to produce plasmolysis of the cells of *Spirogyra nitida* a solution of glycerol was necessary of considerably higher concentration than that isotonic with a solution of sucrose which would produce plasmolysis. This method was later extended to a large number of different cells, and it was found that glycerol is very generally permeable to plant cells, although there are exceptions. Thus de Vries showed that the cells of the bud scales of *Begonia manicata* are impermeable to glycerol and also to

urea (de Vries, 1889 *a, b*). Later Overton (1895) tested thè plasmo-
lytic action of a large number of substances, and with many was
unablè to bring about plasmolysis at all, notaĺy with the alcohoĺs
of the fatty series, various narcotics (ether, chloral hydrate) and other
organic substances.

"*Permeability Coefficients.*" By determinations of the concentra-
tion of penetrating substances required to produce plasmolysis, and
the concentrations of a non-penetrating substance required tò pro-
duce plasmolysis of the same cells, Lepeschkin (1908 *a*) and Tröndle
(1909, 1910) claim to be able to obtain values termed "permeability
factors" by the former and "permeability coefficients" by the latter,
which are measures of the permeability of the cell. Thus, let it be
supposed that the osmotic pressure of the solution of a non-penetrating
substance that just brings about plasmolysis is P_0 while the osmotic
pressure of the solution of the penetrating substance required to effect
plasmolysis is P. The osmotic pressure of the solution of penetrating
substance isotonic with the solution of non-penetrating substance is
also P_0. As P is greater than P_0, it follows that the isotonic coefficient
of the penetrating substance determined by plasmolysis will be less than
that obtained by direct measurements of the osmotic pressure with
a perfectly semi-permeable membrane, or that calculated from deter-
minations of the osmotic pressure from other physico-chemical data.

If i is the true isotonic coefficient calculated from physico-
chemical data and i' is the isotonic coefficient obtained by the plasmo-
lytic method with the particular cells and substance under investiga-
tion, the permeability factor or permeability coefficient is given by

$$\mu = 1 - \frac{i'}{i}$$

and this permeability factor or coefficient is held to be proportional
to the permeability of the cells employed to the particular substance.

It is difficult to understand how such considerations can lead to
results having any definite quantitative significance. We have ob-
served that the reason why a higher osmotic concentration of a sub-
stance that penetrates is required to effect plasmolysis than of one
which does not, is because some of the dissolved substance immedi-
ately enters the cell and so increases the osmotic concentration of
the cell sap. The difference between the osmotic concentration of a
penetrating and a non-penetrating substance required to bring about
incipient plasmolysis is therefore a measure of the amount of sub-
stance which has entered the cell between immersion in the solution
of the penetrating substance and the moment of observation. The

entrance of substance commences the instant the cells are placed in the solution but no doubt takes some time, and probably some very considerable time, before equilibrium is attained. The apparent iso-tonic coefficient will therefore vary with the time that has elapsed before the plasmolysed cells are observed. This was indeed recognised by Tröndle, but he appears to regard the lowering of the apparent isotonic coefficient which must result with increase in the osmotic concentration of the cell as indicating an increase in permeability. If the opinions of Lepeschkin and Tröndle have been interpreted aright, the so-called permeability factors or coefficients could only be used as quantitative measures of intake if the apparent isotonic coefficients were determined after a definite and constant time of immersion of the cells in the solution. Even then exosmosis is neg-lected, and it must be assumed that the substance does not react with a cell constituent. Defining the permeability of the cell to dis-solved substance as the quantity of the substance diffusing through unit area of the cell membranes in unit time when there is unit difference of concentration of the substance between the external solution and the cell sap, it is clear that the method of permeability coefficients could not be used without modification and elaboration to give measures of the permeability of cells to dissolved substances.

Recovery from plasmolysis. If a cell is plasmolysed by a hyper-tonic solution of a penetrating substance, the entrance of the sub-stance into the cell, provided no complicating factors are present such as removal of the substance into an osmotically inactive state, or excessive exosmosis, will increase the osmotic concentration of the cell sap, and in consequence the cell will slowly recover from plasmo-lysis. Recovery from plasmolysis can thus be used as a test of per-meability, and has indeed been one of the favourite methods of in-vestigating permeability since it was first introduced.

This method appears to have been first used by de Vries (1871 *a*) who showed the permeability of beet root cells to sodium chloride by its means. Later Klebs (1887, 1888) recorded the permeability of cells of *Zygnema* to glycerol on account of the recovery of cells of this alga from plasmolysis with a 10 to 20 per cent. solution of this sub-stance. Since then a number of attempts have been made to obtain quantitative data with regard to the entrance of salts and the per-meability of cells by means of plasmolytic data, and various methods have been devised which will now be described.

Tröndle's Method. Tröndle (1920) has attempted to adapt this method to the measurement of the quantity of a substance absorbed

in a certain time. Pieces of tissue assumed to be similar are placed in solutions of the substance of various concentrations, and the tissue examined at different times from the commencement of the experiment. The concentration of the solution in which plasmolysis is just visible is noted at each time. Then if after the lapse of 10 minutes this concentration is xN and after the lapse of a further 10 minutes yN, it is assumed that a quantity of salt has entered the cells of the tissue sufficient to raise the concentration of the substance in the cell sap by a quantity $(y - x) N$. This method, like other work of the same author, assumes that the external concentration of the substance has no influence on the rate at which it is absorbed, which indeed Tröndle emphasizes is the case in salt absorption, an assumption the evidence in favour of which is very doubtful, and the evidence against which is very strong.

Lepeschkin's Method. An attempt to obtain data with regard to the entrance of glycerol into cells of *Spirogyra* was made by Lepeschkin (1908 *a*) in the following way. Cells were plasmolysed in a solution of sucrose, which is assumed not to enter the cells, and left in the solution for an hour, when their volume was measured. The sugar solution was then replaced by a solution of glycerol and the volume again measured after 0·5 hour and again after a further two hours in the glycerol solution. Then if the volume of the cell after the first 0·5 hour is V_2 and after a further two hours V_3, the quantity of glycerol which enters the cell in two hours is

$$\frac{C\ (V_3 - V_2)}{1000}\ \text{gm.-mols.},$$

where C is the concentration of the glycerol solution in gm.-mols. and the volume is measured in c.c.

Lepeschkin defines the permeability as the ratio of the number of gram-molecules of the substance which pass through unit area of the cell membrane in an hour to the difference in concentration of the substance on the two sides of the membrane. If then the mean surface of the cells is S, the permeability is

$$\frac{V_3 - V_2}{1000\,S \left[1 - \dfrac{(V_3 - V_2)\,(V_3 + 4V_2)}{8V_2 V_3} \right]},$$

for the mean difference in concentration between the external and internal concentrations of the substance (glycerol) is

$$C - \frac{1}{2} \left[\frac{(V_3 - V_2)\,C}{4V_2} + \frac{(V_3 - V_2)\,C}{V_3} \right],$$

that is $$C\left[1 - \frac{(V_3 - V_2)(V_3 + V_2)}{8V_2 V_3}\right].$$

In this way Lepeschkin found values for the rate of entrance into the cells varying between 67×10^{-9} and 183×10^{-9} gram-molecules of glycerol per sq. cm. per hour in the case of glycerol penetrating into *Spirogyra*. These numbers are taken as direct measures of the permeability.

Values of the "permeability factor" were also obtained from calculations of the apparent isotonic coefficient of glycerol made at the same time on the same cells as those used for direct determinations of the "permeability."

This was done in the following way. It will be recalled that the cells were first plasmolysed in sucrose (of concentration C_0) and the volume of the cells measured after an hour. This volume is V_1. The cells were then transferred to a solution of glycerol of concentration C and the volume measured again after the lapse of $0 \cdot 5$ hour and again after the lapse of a further two hours, the two volumes being respectively V_2 and V_3. Since the cell is increasing in volume at the rate of
$$\frac{V_3 - V_2}{2}$$

per hour, its volume immediately after addition of the glycerol should have been
$$V_2 - \frac{V_3 - V_2}{4}.$$

Hence the concentration of glycerol apparently isotonic with the sucrose used is
$$C_x = \frac{V_2 - \dfrac{V_3 - V_2}{4}}{V_1} C$$

and the apparent isotonic coefficient of glycerol is
$$1 \cdot 88 \frac{C_0}{C_x},$$

since $1 \cdot 88$ is the isotonic coefficient of sucrose.

Values of the "permeability factor" calculated in this way were found to be roughly proportional to the actual determinations of the permeability. Considering the very doubtful value of "permeability factors" it is surprising that the agreement should be as close as that actually found.

Fitting's Method. Fitting (1915) has devised a method for estimating the intake of salts by the rate of deplasmolysis, using cells of

Tradescantia (Rhœo) discolor. Similar cells which may be supposed to have the same osmotic concentration were plasmolysed in solutions of a salt of different concentrations. If the salt enters the cells, the latter deplasmolyse. Now a stronger solution will produce a greater degree of plasmolysis than a weaker solution, so that as cells that have been plasmolysed in the stronger solution deplasmolyse, a stage will be reached in which the degree of plasmolysis is the same as that originally produced in the weaker solution. During the time that elapses between the commencement of deplasmolysis and the reaching this less degree of plasmolysis, a quantity of salt must have entered the cells sufficient to increase the concentration of the salt in the cells by the difference in concentration between the weaker and stronger solutions. The degree of plasmolysis is measured by a rough estimate of the proportion of cells in the preparation which are plasmolysed, as, for example, one-half, or three-quarters. As there is no great precision in this mode of determining the degree of plasmolysis, Fitting's method cannot be regarded as an exact one. It also does not take account of exosmosis from the cells, which Fitting supposes is eliminated or rendered negligible by a preliminary treatment with water, a conclusion which is probably not correct. Results obtained by its means by Fitting and Tröndle will be dealt with in the next chapter.

Höfler's plasmometric method. The principle of the method used by Höfler (1918 *a, b,* 1919) is the same as that of the preceding, but the degree of plasmolysis is determined by actual measurements of the cells examined, so that the rate of deplasmolysis is followed in individual cells. The cell is plasmolysed by a decidedly hypertonic solution, the degree of plasmolysis being p_1. After the lapse of a time t the degree of plasmolysis is p_2. Then if the concentration of the external solution is C, and the osmotic concentrations of the cell corresponding to the two degrees of plasmolysis are respectively C_1 and C_2, we have

$$C_1 = Cp_1$$

and

$$C_2 = Cp_2,$$

whence

$$C_2 - C_1 = C(p_2 - p_1).$$

Since the change in osmotic concentration of the cell in unit time is a measure of the rate of intake of the dissolved substance, the rate of intake can be determined from the concentration of the external solution and the change in the degree of plasmolysis.

Höfler's method, like those of Lepeschkin and Fitting, neglects the possible effects of exosmosis. Apart from this and the assumption

that the substance diffuses into the cells and there undergoes no reaction with the cell contents, the method seems to be a sound one for the determination of the absorption of dissolved substances. Höfler, however, like so many other observers, confuses the rate of intake with permeability. Thus, he defines permeability as the quantity of substance entering the cell in unit time, thus neglecting the fact that the rate at which the substance enters the cell depends not only on the permeability of the cell membrane, but also on the difference in concentration of the substance in the external solution and in the cell sap. This does not matter so much, as Höfler makes it perfectly clear what he is measuring, so that there is no obscurity. His results will be dealt with later.

11. THE DETERMINATION OF PERMEABILITY BY CHANGES IN WEIGHT OR VOLUME OF TURGID TISSUES

The principle of this method is much the same as that of the plasmolytic method. If turgid tissues are immersed in a solution of a substance strong enough to produce a contraction in the volume of the cells, but not concentrated enough to produce plasmolysis, the penetration of the dissolved substance into the cells of the tissue will bring about a gradual increase in volume (and consequently in weight) of the tissues. This method has been employed to test the entrance of salts and other substances into animal tissues and cells, *e.g.* blood corpuscles, by Köppe (1895 *a, b*), Kozawa (1913, 1914) and others. For work with plant tissues the method has been developed by Lundegårdh (1911) who used it to study the entrance of salts into roots of *Vicia Faba*. For this purpose the roots were placed horizontally in a small cell under the microscope and changes in length of the root measured. The roots were first treated with a solution of the salt under investigation which produced a contraction in length. As the salt entered the tissue the root gradually increased in length. The reciprocal of the time taken for the root to increase in length from 25 per cent. to 75 per cent. of the total increase it underwent was taken as a rough measure of the rate of entrance of the salts.

12. TISSUE TENSION METHOD

A tissue tension method for studying permeability to dissolved substances has been described by Brooks (1916 *c*). The experimental procedure is as follows. Similar strips of the peduncle or midrib of dandelion (*Taraxacum officinale*, Weber) were fixed at one end between

the two halves of a partially split rubber stopper and the strips held horizontally so that the free ends could move in a horizontal plane. The strips so held were immersed in 20 c.c. of a solution of the substance to be examined in which the strips underwent no appreciable change in curvature. The concentration of the solution was then increased by the addition of a known small volume of a molecular solution of the salt. As a result the strip of tissue underwent a decrease in curvature, which soon ceased and was followed by a slow increase in curvature. The time that elapsed between the increase in concentration and the instant that the strip regained its original curvature was called the time of recovery. Immediately on recovery the concentration of the solution was again raised and the time of recovery again noted. The process was repeated several times and in this way a series of times of recovery was obtained. An empirical value for the rate of penetration was obtained by dividing the change in concentration by the time of recovery. Curves were then plotted between these rates of penetration and the times that had elapsed between the first immersion of the tissue in the solution and the middle of each recovery time. These curves are supposed to represent the rate of penetration of the salt into the tissue.

Brooks appears to regard the values he obtains for rate of penetration as measures of permeability. He has good grounds for doing this, as the difference in concentration between external solution and cell sap with regard to the substance under investigation is the same at the moment at which each recovery period commences. But the effects of exosmosis are not considered, nor are the possibilities of any reactions between the penetrating substance and the contents of the cell sap. Also the results obtained during the first 15 or 20 minutes were very irregular, and were therefore disregarded. The method in its present form is thus not free from objection.

13. DIFFUSION METHOD

This method has also been described by Brooks (1916 *a*, 1917 *b*). Disks cut from the thallus of *Laminaria Agardhii* were placed between two short pieces of glass tube applied to the two sides of the tissue so as to make a watertight join. The lower piece of tube was closed below by a rubber tube and clip. The cell thus formed was filled with a salt solution having the same conductivity as sea water, or with sea water itself, and the upper tube with a known quantity of a solution of the same composition but of one-half the concentration. The

rate of increase of the electrical conductivity of the solution in the upper tube and the decrease in the conductivity of the solution in the lower tube was taken as a measure of the rate at which the salt or salts in the lower cell passed through the tissues, and therefore as a measure of the permeability of the tissue. To eliminate errors due to exosmosis from the cells and diffusion from the intercellular spaces of the tissue, control experiments were made in which the more dilute solution was contained in both upper and lower tubes. The average conductance of the solution in the upper tube of the controls at the end of the experiments was taken as the standard, and the average conductance of the solutions in the experimental tubes "divided by this figure in order to obtain the percentage which expresses their gain as compared with the control." By this method it is stated that "the figures which were obtained in this manner measure the amount of salt which had passed through the tissue, while the errors due to exosmosis from the protoplasm as well as those due to diffusion from the intercellular substance are eliminated."

As disks of tissue vary in thickness, errors arising from this cause were eliminated by first performing an experiment with sea water and then using the same disk for an experiment with one of the salts examined. The rate of change of conductivity during the experiment with the salt solution was then divided by the rate of change in conductivity during the preliminary period when sea water was used. Experiments were also made with dead tissue.

The principal objection that can be raised to this method is that there is no guarantee that the salt which diffuses into the cells on the lower side of the *Laminaria* thallus is the same salt which diffuses out from the cells on the upper side of the thallus. Possible combinations between salt and cell constituents are completely neglected. In short, the complexity of the system is disregarded, and the disk of tissue, several cells in thickness, is regarded as a simple membrane. How far this is justifiable is doubtful.

The method has also been used to examine the permeability of cell walls, bulb scales of *Allium* being employed for this purpose (Brooks, 1917 *c*).

14. The Determination of Permeability to Dissolved
‣ Substances by Measurement of the Electrical
Conductivity of Living Tissues

A method for the measurement of the electrical conductivity of living tissues has been elaborated by Osterhout (1912 *a*, 1913 *a*, 1914 *j*, 1918 *c*, 1919 *a*, 1921) who regards the electrical conductivity of a tissue as a measure of the permeability of the protoplasm. Most of Osterhout's measurements have been made with *Laminaria* thallus, but in the latest of the papers cited above apparatus is described suitable for use with other tissue, while a few experiments have been made with animal tissue (1919 *c*). The experimental material most usually employed consisted of a pile of about 80 to 200 circular disks cut from the thallus of *Laminaria Agardhii* and arranged to form a cylinder like a pile of coins, the column of disks being kept in position by means of glass rods. At each end of the cylinder, and separated from it by a small length of solution, was a platinum electrode coated with platinum black contained in an electrode holder of hard rubber. The measurements of electrical conductivity were made by means of Kohlrausch's method[1].

The question at once arises how far the electrical conductivity of tissue is a measure of the permeability of the protoplasm contained in its cells. This question has been discussed by Stiles and Jørgensen (1918) who have pointed out various difficulties which stand in the way of accepting the electrical conductivity as an exact measure of permeability. The electrical conductivity of a piece of tissue will depend on the conductivity of a number of different phases in the tissue, and we must consider the possible changes in all these phases when the conductivity alters. An increase in conductivity of living tissue is interpreted by Osterhout as an increase in the permeability of the protoplasm to ions. Now an increase in conductivity could be brought about in a number of ways apart from an alteration in the permeability of the protoplasm. Thus a change in the state of aggregation of the contents of the cell wall by which the content or nature of electrolytes was altered, as, for example, by diffusion or by breaking down of complex compounds, would alter the conductivity. This is by no means to be ruled out as a possibility having regard to the complexity of the cell wall (cf. Chapter VII). Experiments with dead tissue (cf. Osterhout, 1918 *a*), in which it is shown that dead tissue

[1] See also W. J. V. Osterhout, *Injury, Recovery and Death in Relation to Conductivity and Permeability*. Philadelphia and London, 1922.

behaves differently from living tissue, are not convincing evidence that the changes observed in living tissue are not in any way connected with the cell wall, for in the present state of our knowledge it is not altogether certain that the cell walls of dead tissue are in the same state as those of the tissue before death. Even if the cellulose should be unaltered by the method of killing the tissue, other constituents of the cell wall and its general physical properties may be changed.

But changes in the content and nature of the electrolytes in the protoplasm and cell sap are likely to contribute much more than changes in the cell wall to alterations of the conductivity. Anything, such as diffusion, which brought about an increase in the quantity of free electrolytes in the protoplasm or cell sap, would raise the conductivity, and so would the breaking down of large complex molecules to smaller ones which undergo more ionisation or which give more mobile ions than the complex molecules from which they are derived. Such a change in the protoplasm might well involve an increase in its permeability, but the mere fact that its conductivity was higher, due to its higher content of ions or of more mobile ions, is not in itself to be interpreted as a proof of greater permeability.

How difficult it is to interpret the results obtained by the conductivity method may be exemplified by reference to an experiment made by Osterhout (1918 b) in which Laminaria tissue was transferred from artificial sea water containing sodium chloride to a similar solution having the same conductivity but in which lithium chloride was substituted for sodium chloride. This transference resulted in a fall in the resistance of the tissue, while on transferring the tissue back to the original medium the resistance rose.

Osterhout states that these effects are clearly due to diffusion. He says that "the smaller molecules of LiCl diffuse in faster than NaCl can diffuse out, causing a temporary excess of salt, which lowers the resistance." Now whatever the reason for the change in resistance the facts cannot be as Osterhout supposes, for the coefficient of diffusion of lithium chloride (in spite of its "smaller molecules") is slightly lower and not higher than that of sodium chloride as shown for diffusion in water by Öholm (1905) and for diffusion in gels by the present writer[1]. It is nevertheless encouraging that Osterhout acknowledges that factors other than permeability of the protoplasm can affect the electrical conductivity of tissue.

[1] W. Stiles, "The Indicator Method for the Determination of Coefficients of Diffusion in Gels, with Special Reference to the Diffusion of Chlorides." *Proc. Roy. Soc.* A, **103**, 260–275, 1923.

In short, therefore, in the opinion of this writer, while changes in the conductivity of tissues may be correlated with similar changes in the permeability of the tissues, there appear to be so many other possible explanations of changes in electrical conductivity of tissue, that conclusions relating to permeability changes based on the assumption that the electrical conductivity is a quantitative measure of permeability to ions must be accepted with very great caution.

This concludes our review of the methods that have been devised for the study of absorption of dissolved substances and the permeability of the cell to such substances. These methods are very varied and many of them decidedly ingenious. From this survey it is obvious that reliable data are often readily obtainable with regard to qualitative relations in permeability, and the more important qualitative results have been recorded in the course of this chapter. But when we consider quantitative relations in absorption and permeability the case is very different. Although many results have been recorded professing to give quantitative data with regard to intake of dissolved substances and the permeability of cells to such substances, yet in most cases the presence of disturbing factors renders the results open to criticism. The reason for this is to be found in the complexity of the system involved, combined with the very general assumption that the conditions are simple. Thus, to take only one case, that of the plasmolytic method, it is assumed in this method that the substance which enters the cell passes through a protoplasmic membrane and then accumulates in the cell sap as such, and that there are no disturbing factors to complicate this simple arrangement. Exosmosis, which certainly takes place, is neglected. The possibility of combination of the diffusing substance with a cell constituent or its adsorption with such a cell constituent, which we have seen may certainly take place in certain cases, is also neglected.

In the next chapter, where the quantitative relations of the cell to dissolved substances are considered, it will therefore be necessary to subject the data dealt with to some scrutiny, in order to have as clear an idea of what the results obtained really mean, as the difficulty and complexity of the subject make possible.

CHAPTER XII

QUANTITATIVE RELATIONS IN THE PENETRATION OF DISSOLVED SUBSTANCES INTO PLANT CELLS

WHILE qualitative tests of the penetration of dissolved substances into plant cells have yielded results of considerable interest, we can hardly expect to be able to formulate the laws governing the passage of substances into and out from the cells without adequate quantitative data. These data we most certainly do not yet possess; nevertheless what information we have is of great interest and is sufficient to show that the simple osmotic view of the plant cell is a very inadequate hypothesis and is incapable of affording a complete explanation of the cell in regard to its relations to dissolved substances. Without further preface various aspects of the quantitative relations of plant cells will be dealt with in the following sections of this chapter.

THE UNEQUAL ABSORPTION OF THE IONS OF A SALT BY PLANT TISSUE

In earlier work on the absorption of salts by plant tissue it was assumed that salts were absorbed as such by plants. Of late years, however, it has come to be recognised that there may be an unequal absorption by plant tissue of the two ions of a single salt. In the light of recent knowledge certain observations of long standing become easily explicable on this ground, such as the acidity or alkalinity developed in some water culture solutions in which plants have been growing for a time.

If unequal absorption of ions takes place there are necessary consequences of such a phenomenon. The penetration of an excess of one ion into the tissue cannot, on account of the attraction of the oppositely charged ions, take place without the replacement of this excess by an equal quantity of another ion carrying the same charge.

This replacement can take place in two ways. In the first way an equivalent quantity of hydrogen-ion or hydroxyl-ion, as the case

may be, can appear in the external solution, this hydrogen-ion or hydroxyl-ion being derived from the solvent, in which case the excess of the absorbed ion is accompanied into the tissue by an equivalent quantity of hydroxyl-ion or hydrogen-ion according as the excess of absorbed ion carried a positive or negative charge. In the second way an equivalent quantity of some other ion carrying the same charge as the ion absorbed in excess, diffuses out from the tissue.

To take a definite example, let us suppose that a particular tissue immersed in a solution of potassium nitrate absorbs the nitrate ion in excess. Either an equivalent quantity of hydroxyl ions appears in the solution to balance the excess of potassium ions left there, the excess of nitrate being accompanied into the tissue by an equivalent quantity of hydrogen-ions; or an equivalent quantity of some anion or anions, as, for example, sulphate, malate, citrate, diffuses out from the tissue. If it can be shown that an unequal absorption of ions takes place a complete view of the process can only be obtained by determining the quantities of hydrogen, hydroxyl or other ions that appear in the external solution.

Unequal absorption of ions by isolated tissues. Direct observations on the intake of the different ions of a salt by isolated tissues are few. Some have, however, been made by Meurer (1909) on the absorption of a number of salts by slices of beetroot and carrot 3 mm. in thickness. A few observations have also been made by Ruhland (1909 *b*) on beetroot in the form of slices 3 mm. and 1 mm. in thickness. Some of the results of these authors are summarised in the following table. The absorption in every case is given as the proportion of the concentration of ion absorbed to the concentration of the ion in the external solution.

TABLE XXX

Absorption of Ions of a Number of Salts by Plant Tissue

Tissue	Salt	Concentration of solution	Duration of absorption in days	Relative Absorption		Observer
				Kation	Anion	
Carrot	KCl	$N/15$	2	0·374	0·287	Meurer
,,	NaCl	$N/12$	2	0·411	0·258	,,
,,	CaCl$_2$	$N/14$	2	0·270	0·229	,,
,,	KNO$_3$	$N/20$	4	0·524	0·570	,,
Beetroot	CaCl$_2$	0·4 %	2	0·258	0·0354	Ruhland

These results, as far as they go, give clear indications of an unequal absorption of ions by the storage tissues examined. The

observations are, however, not numerous, and under the circumstances it would be unwise to draw too far reaching generalisations from them. Indeed, no suggestion of a general rule is apparent from these results. Thus, with carrot the kations of most of the chlorides examined were absorbed to a somewhat greater extent than the chlorion in each case, while with beetroot this difference is very much emphasised in the case of calcium chloride, about seven times as much calcium ion being absorbed as chlorion in the same time. On the other hand carrot absorbed more chlorion than kation from a solution of magnesium chloride (cf. Table XXXI), and slightly more nitrate than potassium from a solution of potassium nitrate.

That this unequal absorption of ions is a property of living cells and not of dead tissue is shown by Meurer's results with living and dead carrot tissue immersed in solutions of magnesium chloride (Table XXXI). Whereas with living tissue about 32 per cent. more anion was absorbed than kation during four days, with dead tissue equivalent quantities of the two ions were absorbed.

TABLE XXXI

Absorption of the Ions of Magnesium Chloride by Dead and Living Carrot

Concentration of solution	State of tissue	Duration of absorption in days	Relative absorption by	
			Kation	Anion
$N/24$	Living	2	0·327	0·336
"	"	4	0·286	0·377
$N/22$	Dead	2	0·958	0·950
		4	0·953	0·953
$N/95$	Living	2	0·563	0·774
		4	0·577	0·895
$N/105$	Dead	2	·—	0·950
"	"	4	—	0·869

Unequal absorption of ions by whole plants. The unequal absorption of ions by *Cucurbita Pepo* was investigated by Pantanelli and Sella (1909). The plants were grown as in water-culture, 16 to 21 individuals being placed in each vessel. After the roots had been surrounded by conductivity water for two days, the plants were transferred to the experimental solutions for a definite number of days, after which time the dry weights of roots and shoots were determined and the external solution analysed for both ions of the experimental salts. The results obtained are summarised in Table XXXII.

TABLE XXXII

Absorption of Ions by the Roots of Living Plants of *Cucurbita Pepo*.
(Data from Pantanelli and Sella)

Salt	Concentration in gm. mols. per litre	Duration of experiment in days	Absorption in mg. ions	
			Kation	Anion
Potassium chloride	0·03	6	23·38	30·68
Calcium chloride	0·02	14	0·00	51·39
Potassium sulphate	0·0188	6	11·6	18·07
Calcium sulphate	0·0165	19	0·00	1·98
Potassium acid phosphate	0·02	10	1·15	49·04
Calcium phosphate	0·032	12	1·10	78·93

These results exhibit very clearly the very great differences which may exist between the quantities of kation and anion absorbed by the roots of living plants in the same time. In all these experiments the anion was absorbed in excess of the kation, sometimes very greatly in excess. Calcium was absorbed either to a very slight extent, or not at all.

This work was later extended by Pantanelli (1915 *a, b, c*) to a number of other species, including freshwater plants (*Elodea canadensis, Azolla caroliniana*), higher land plants (*Allium Cepa, Phaseolus multiflorus, Cicer arietinum, Vicia Faba, Lupinus albus*), yeast of barbera and marine algæ (*Gigartina acicularis, Cryptonemia Lomation, Phyllophora nervosa, Dictyota dichotoma, Ulva lactuca, Valonia utricularis*). The experiments were carried out at temperatures between 15° and 20° C. A wider range of salts was also employed. Although some cases were observed in which equivalent quantities of the two ions of a salt were absorbed, in the vast majority of cases the absorption of the kation and anion was unequal. A number of Pantanelli's results are collected in Table XXXIII.

Pantanelli's results, of which those shown here are only a small selection, show that unequal absorption of the ions of a salt is an almost universal phenomenon, at any rate in the concentrations used. Slight unequal absorption of the constituent ions of calcium chloride by beet, carrot and maize after the roots had been immersed in the solution (initially about 0·04 N) for periods varying from 8 to 39 days, was also observed by Johnson (1915). Hoagland (1918) found that barley absorbed more nitrate than sodium from solutions of sodium nitrate, but that the two ions of potassium chloride were absorbed by this plant in equivalent proportions.

TABLE XXXIII

• Absorption of Ions by Living Plants. (Data from Pantanelli)

Species	Salt	Concentration in gm. mols. per litre	Duration of experiment in hours	Absorption in mg. ions Kation	Anion
Elodea	Calcium chloride	0·05	2	3·0	0·41
canadensis	Potassium sulphate	0·05	2	3·44	0·29
,,	Ammonium sulphate	0·05	2	0·29	2·05
Azolla	Calcium chloride	0·05	2	0·26	0·00
caroliniana	Potassium nitrate	0·05	2	1·89	0·91
,,	Aluminium nitrate	0·0125	2	0·16	0·23
,,	Potassium sulphate	0·025	2	2·67	2·5
,,	Ammonium sulphate	0·025	2	0·17	2·28
,,	Magnesium sulphate	0·025	2	3·58	2·48
,,	Ferrous sulphate	0·025	2	0·76	0·61
,,	Aluminium sulphate	0·0125	2	0·95	0·062
Phaseolus	Calcium chloride	0·01	8	1·18	1·50
multiflorus	Barium chloride	0·01	8	0·04	0·77
Cicer	Potassium chloride	0·025	8	0·35	0·23
arietinum	Calcium chloride	0·025	8	0·025	0·14
,,	Potassium nitrate	0·025	8	2·74	1·95
,,	Aluminium nitrate	0·0125	8	0·68	2·31
Ulva lactuca	Calcium chloride	0·025*	2	3·29	2·79
,,	Potassium sulphate	0·025*	2	1·48	0·18

* 10 c.c. of 0·25 M salt + 90 c.c. sea water.

The unequal absorption of the ions of calcium chloride by *Pisum sativum* and *Zea Mays* was shown by Miss Redfern (1922 *a*), who examined the influence of the concentration of the salt on the degree of inequality of absorption by the former species. She found that the more dilute the solution the less the divergence between the absorption of the two ions. Her results for the edible pea are shown in tabular form below (Table XXXIV).

TABLE XXXIV

Influence of Concentration on the Absorption of the Ions of Calcium Chloride by the Roots of Living Plants of *Pisum sativum*.

(Data from Redfern)

Initial concentration of solution	Percentage absorption after 36 hours Calcium	Chloride
0·1 N	17·74 ± 1·376	3·578 ± 0·506
0·01 N	19·61 ± 2·33	12·47 ± 1·66
0·001 N	23·10 ± 5·30	15·09 ± 3·736

It appears likely that the excess absorption of one ion is accompanied in some cases by the solution developing an equivalent quantity of hydrogen or hydroxyl ions so that the solution becomes

acid or alkaline, while in other cases the equality of positively and negatively charged ions in the external solution is maintained by exosmosis of ions from the plant cells. The acidity and alkalinity that may be developed in water culture solutions, and which has been noticed from the time of Knop onwards, can be explained as a case of the former happening. On the other hand, Hoagland (1918) found that the culture solution in which barley was growing maintained a neutral reaction, and, if the reaction was acid to start with, the solution became neutral after contact with the roots for a time.

Pantanelli considered that the unequal absorption of the two ions resulted in the development of acidity or alkalinity at first, but that after a time this might disappear and the excess of absorbed ion become balanced by excretion of oppositely charged ions from the tissue.

Miss Redfern found that the calcium chloride solutions used in her experiments in which the calcium ion was absorbed in excess remained approximately neutral throughout the experiment, while tests of the external solution showed that magnesium and potassium ions had diffused out of the tissue. Therefore the excess of calcium ion absorbed is replaced in this case by the diffusion out of the tissue of ions carrying a similar charge, and not by hydrogen ions from the water of the solution.

Stoklasa, Šebor, Týmich and Cwacha (1922) also conclude that the absorption of aluminium and ferric ions by the roots of living plants of *Eriophorum vaginatum, Phragmites communis* and *Carex riparia* is accompanied by the excretion of calcium, magnesium and sodium ions. Their conclusion is based on water culture experiments in which the analysis of the solutions was made after the experimental plants had been growing in them for 13 days. Since the aluminium ion is absorbed much more rapidly than the anion, this excretion of other kations must necessarily take place if the solution does not become highly acid or alkaline.

The Position of the Equilibrium Attained in the Intake of Dissolved Substances by Plant Cells

It is a remarkable fact, and one indicating how little the complexity of the problems involved in the phenomena of permeability and absorption have been realised by workers in these fields, that scarcely any of those who have attempted to obtain quantitative data with regard to permeability have concerned themselves with the equilibrium attained in the passage of dissolved substances into

plant cells. Yet it is obvious that a determination of the rate of intake of a substance can give no indication of the permeability of the cell unless the position of the equilibrium in the intake is known. Some few writers have, indeed, had a clear conception of this. Thus Pfeffer pointed out that a cell might be permeable to a dye and yet the intake of the dye might be unobservable because no accumulation of the dye took place in the interior of the cell. Yet in employing the favourite method used in recent work for determining the rate of intake of dissolved substances, that is, the plasmolytic method, investigators most usually do not consider the position of equilibrium at all, while the possibility of an equilibrium with different concentrations of the substance inside and outside the cell, is never considered. Quite usually the rate of intake is regarded as a measure of permeability. This would only be so if the difference in concentration between the external solution and the cell sap remained constant, which, according to the theory on which the method is based, is not the case. Lepeschkin, it is true, takes account of the change in concentration difference between external solution and cell sap as deplasmolysis proceeds, and so in treating his experimental data, is at any rate consistent with the theory on which he works.

But is it correct to assume that the dissolved substance diffuses into the cell until there is equality of concentration of this substance inside and outside the cell? Pfeffer's work with dyes showed most undeniably that this is not the case with the majority of dyes examined by him, while it is equally certain that many substances occur normally in the plant in considerably higher concentration than they do in the external medium from which they were obtained. Wherever chemical combination or adsorption takes place in the interior of the cell it is obvious that the assumption made in the plasmolytic and some other methods, that the penetration of the dissolved substance into the cell can be regarded simply as the passage through a membrane into a medium which does not react with the substance, is unsound. It is therefore very necessary that the evidence available with regard to the position of this equilibrium should be examined.

We may notice in the first place that a number of observations are on record which indicate that the concentration of a substance inside a cell can remain greater or less than its concentration in the external medium. With animal cells the case of blood corpuscles and serum may be cited, and with plant cells the observations of Wodehouse (1917) on the concentration of various substances in the

cell sap of *Valonia* (see Chapter XI)[1]. The explanation generally offered of this phenomenon is that the cell membranes are impermeable to the substance in question, so that it can exist in different concentrations on the two sides of the membrane. This cannot be the explanation of Pfeffer's results with dyes, where the accumulation of the dye in the cell sap is accounted for by chemical combination of the dye with a cell constituent to form a non-diosmosing compound. But some of those who have employed the plasmolytic and other methods have curiously enough neglected the possibility of such a complication in the intake of the substances they have examined.

We must particularly notice in this connexion the opinion of Moore and Roaf (1908) and Moore, Roaf and Webster (1912), who rejected the theory of a membrane impermeable to crystalloids as the explanation of the permanent difference in concentration on the two sides of the membrane, and who held that the observed results could be adequately, and more satisfactorily, explained as due to adsorption of the penetrating substance by cell constituents or to chemical combination between the penetrating substance and some cell constituent or constituents in the same way as Pfeffer supposed the accumulation of dyes takes place.

The absorption ratio. Actual determinations of the position of equilibrium attained in the penetration of dissolved substances are not numerous, and in practically no case is the method of determining it free from every objection. Such as they are, however, the existing data do provide almost overwhelming evidence to the effect that in no observed case is the position of equilibrium in the intake of any substance necessarily one of equality of concentration in the external solution and the cell sap.

Nathansohn (1903) made chemical analyses of the external solution and the expressed sap of the marine alga *Codium* after immersion for some days in solutions of sodium nitrate. Further experiments were carried out by the same author (1904 *a, b*) on the same lines with slices of tubers of *Dahlia* and *Helianthus tuberosus* and of the root of beet, a number of salts being used. Nathansohn's observations were later extended by Meurer (1909).

The relation between the final internal and external concentrations can best be expressed by the ratio of the first quantity to the second. To this ratio, that of final internal to final external concentration, the name *absorption ratio* has been given by Stiles

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[1] See also the very definite evidence on this matter provided by the recent work of Hoagland, Davis and Martin on *Nitella*. (D. R. Hoagland, A. R. Davis and J. C. Martin, "The Composition of the Cell Sap of the Plant in Relation to the Absorption of Ions." *Journ. Gen. Physiol.* **5**, 629–646, 1923.)

and Kidd (1919 *a*), who calculated the absorption ratios given by
Nathansohn's and Meurer's experimental results. In Table XXXV
are shown a selection of these absorption ratios.

TABLE XXXV.

Absorption Ratios

Tissue	Salt	External concen- tration	Duration of absorption in days	Absorption ratio	
				Kation	Anion
Codium	NaNO$_3$	0·5 %	4		0·56
,,	,,	,,	10	---	0·68
,,	,,	1·0 %	5	--	0·44
Dahlia	,,	,,	4	0·59	---
,,	,,	,,	6	0·51	
,,	NH$_4$NO$_3$	1·5 %	4	0·32	
Carrot	KCl	$N/15$	4	0·548	0·386
,,	NaCl	$N/12$	4	0·489	0·307

It will be observed that in scarcely any case does the absorp-
tion ratio approximate to unity, although having regard to the fact
that thin slices of tissue were used and that the salts employed
have fairly high coefficients of diffusion, the equilibrium condition
could not be far off at the end of the experiment. This is also indicated
by the value of the absorption ratio at the end of, say, four days,
being practically identical with that at the end of six days in the
case of the absorption of sodium nitrate by *Dahlia* tuber.

It can therefore scarcely be argued that as all these absorption
ratios are less than unity the reason for this is that equilibrium had
not been reached when the experiments were brought to a conclusion.
Moreover, in the case of the absorption of aluminium sulphate by
carrot and other tissues Meurer found so great absorption of the
aluminium ion from a 0·056 per cent. solution that the absorption
ratio with carrot after two days was 11·33 and after four days 16·89.
Of course, aluminium may be a special case, and Meurer himself
thought it was absorbed by the cell walls. However, as we shall see
shortly, there is no reason from the high value of the absorption
ratio to suppose that the behaviour of aluminium is exceptional.

In this connexion the results obtained with dyes are interesting.
Reference has already been made to the work of Pfeffer and others
who have shown that in the absorption of many dyes there is a
considerable "heaping up" of the dye in the tissue. This is chiefly,
though not exclusively, a property of the so-called basic dyes, and
Collander (1921) has shown that a number of sulphonic acid dyes
are only absorbed to a comparatively small extent by many plant
tissues. Some of the absorption ratios found by him are collected
in the following table.

TABLE XXXVI

Absorption Ratios of Sulphonic Acid Dyes.
(Data from Collander)

Species	Tissue	Dye	External concentration in per cent.	Duration of experiment in hours	Absorption ratio
Allium Cepa	Parenchyma of bulb scale	Light green	0·8	46	<0·016
Daucus Carota	Root parenchyma	Orange G	0·8	42	<0·016
Hyacinthus orientalis	Cortex of root	Cyanol	0·1	24	<0·125
,,	Storage cells of bulb scales	,,	0·1	24	<0·125
,,	Parenchyma of peduncle	,,	0·1	24	<0·062
,,	,,	Orange G	0·8	72	<0·0078
,,	Bundle sheath of perianth leaves	,,	0·012	24	>4·0
,,	,,	,,	0·05	24	<0·25
Pisum sativum	Cortex of root	Cyanol	0·8	24	<0·062
Rhœo discolor	Spongy parenchyma	Orange G	0·8	48	<0·062
Spirogyra sp.	—	Cyanol	0·8	50	<0·062
,,	—	Orange G	0·8	90	<0·031
,,	—	Fuchsin S	0·8	66	<0·125

It is clear then that the absorption ratios of dyes may vary within very wide limits, from many times unity in the case of most basic dyes, to less than 0·01 in the case of some tissues immersed in solutions of acid dyes.

With dead tissue the absorption ratios in the case of salts are much nearer unity (cf. Table XXXI). The maintenance of equilibrium with different concentrations inside and outside the cell is thus a property of living tissue.

It is interesting to note that the maintenance of an equilibrium in which the concentration of a substance inside the tissue is apparently different from that outside has also been observed in the case of seeds. Brown and Tinker (1916 b) soaked grains of barley in solutions of aniline, phenol and acetic acid, and determined the extent of the absorption of these substances by analysis of the seeds. They found that aniline and phenol accumulated inside the seeds so that the concentration of these substances inside the seeds was about three times as great as the external concentration. With acetic acid, on the other hand, equilibrium was attained when the concentration of the acid inside the seed was about 80 per cent. of the external concentration, the latter being in the neighbourhood of 40 per cent. (the ratio acetic acid : water being between 0·5 and 0·9).

The influence of external concentration on the position of equilibrium. The observations already recorded suggest that the quantity of substance absorbed by the same tissue from a solution is *relatively* greater the diluter the solution, that is, the absorption ratio increases with dilution. The influence of the concentration of a solution external to plant tissue on the position of the equilibrium attained in the intake of a number of salts has been investigated by Stiles and Kidd (1919 *a*) by means of the measurement of the electrical conductivity of the external solution. Experiments were carried out at constant temperature (20° C.) with carrot root and potato tuber in the form of circular disks 1·8 cms. in diameter and 1 mm. in thickness, 40 such disks being immersed in 100 c.c. of solution. These experiments were all conducted in triplicate. For any series of experiments designed to yield comparable results all the disks used were cut at the same time and allowed to swell in water for a preliminary period. After mixing them well together each set of 40 disks was then taken from the general stock. The bottles used to contain the tissue and solutions were continuously shaken throughout the whole course of the experiment. This is a precaution to ensure regular results, as such treatment prevents the formation in the solutions of gentle diffusion gradients which may be unequal over different disks, especially when some of these lie on top of others.

It has been pointed out by Stiles and Kidd that the decrease in the conductivity of the external solution may be assumed to be approximately proportional to the difference between absorption of the salt and exosmosis from the tissue. If the exosmosis into distilled water is determined and added to the values found in the experiments with salts, numbers proportional to the actual salt intake should be obtained provided there are no complications. That this is so cannot be assumed.

Stiles and Kidd pointed out that the following actions would also bring about a decrease in conductivity and so make the values obtained for absorption in the way indicated, too high.

(1) Reactions between the exudate and external solution by which non-ionised molecules are produced. This can be disregarded as a source of appreciable error having regard to the dilution of some of the solutions and the magnitude of the decreases with higher concentrations.

(2) The exosmosis of non-electrolytes which by their mere presence would reduce the conductivity of the external solutions. The quantity of such substance which diffuses from the tissue is probably negligible.

(3) A lessening of exosmosis as compared with the control on account of the action of the salt on the tissue. In the case of carrot exosmosis into distilled water is small, and if the action of the salt were to reduce it to nothing the results would be affected very little.

Hence the method is not likely to give too high values for absorption. On the other hand the following actions would tend to increase the conductivity and so render the observed measures of the absorption too low.

(1) Increased exosmosis resulting from the action of the salt on the tissues. This, long continued, leads to the death of the tissue (Stiles and Jørgensen, 1917 *a*) and consequently to loss of its turgidity. But at the end of the experiments there was no loss of turgor of the tissues employed, so that it is unlikely the values obtained for absorption are appreciably lowered on this account.

(2) An unequal absorption of the ions of a salt as described earlier in this chapter. In this event it is the approximate absorption of the less absorbed ion that would be measured, for the excess of the more absorbed ion must be replaced by an equivalent quantity of a similarly charged ion, either H or OH from the water of the external solution, or by an ion escaping from the tissue, probably the latter (Redfern, 1922 *a*). Moreover, as the mobilities of the absorbed and replacing ions will be different in all probability, and as a difference in the degree of dissociation may result, the fall in conductivity will only give an approximate value of the absorption. Owing to the possibility of this complication the results of Stiles and Kidd are therefore to be regarded as giving approximate values of the absorption of the less absorbed ions of salts.

In the following table are shown the absorption ratios obtained for the absorption by carrot tissue of various chlorides from solutions possessing initially concentrations ranging from 0·1 *N* to 0·0002 *N*.

The results with each salt are all strictly comparable, but the results with different salts should not be so compared as different batches of tissue were used for the experiments with each salt.

If the logarithms of the final external concentrations are plotted against the logarithms of the final internal concentrations the points in the case of each salt lie approximately on straight lines. In Fig. 13 the logarithms of the final internal concentrations are taken as ordinates and the logarithms of the final external concentrations as abscissæ. It will be observed that the relation is approximately a linear one in the case of each salt.

TABLE XXXVII

Absorption Ratios in the Intake of Various Chlorides by Carrot
Tissue from Solutions of Different Concentrations.
(Data from Stiles and Kidd)

Salt	Initial concentration in normalities	Duration of experiment in hours	Relative final conc. Internal	Relative final conc. External	Absorption ratio
Potassium chloride	0·0002	52	0·60	0·024	25·0
,,	0·002	,,	4·20	0·238	17·6
,,	0·02	,,	11·90	4·882	2·4
,,	0·1	,,	20·50	26·200	0·78
Sodium chloride	0·0002	48	0·56	0·012	46·7
,,	0·002	,,	4·00	0·148	27·0
,,	0·02	,,	10·80	3·990	3·5
,, (dead tissue)	0·02	,,	4·45	5·060	0·88
,,	0·1	,,	17·80	21·550	0·83
Calcium chloride	0·0002	42·5	0·46	0·030	15·3
,,	0·002	,,	1·17	0·420	2·8
,,	0·02	,,	2·50	4·930	0·51
,,	0·1	,,	5·30	22·120	0·24
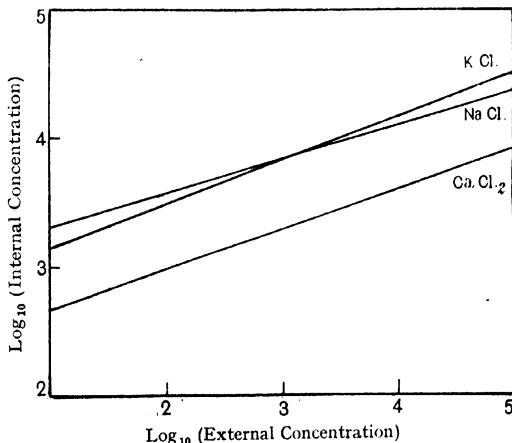
Fig. 13. The relation between final internal and final external concentration in the case of carrot root tissue immersed in certain chlorides. (After Stiles and Kidd.)

Then, if i and e represent the final internal and final external concentrations respectively, the relation between the two is given by the equation

$$\log i - m \log e = \log k,$$

where $\log k$ is a constant.

This equation may be written in the form $i = ke^m$, and this is the adsorption equation. (See Chapter III.)

Although this result does not prove that the intake of salts into the cell is a process of adsorption of the salt by a constituent of the cell, it indicates that this is a possibility. But the results do give clear proof that the absorption of salt by carrot root does not proceed until there is equality of concentration inside and outside the cell. With lower concentrations there is heaping up of salt inside the cell, while with higher concentrations the absorption ratio is less than unity. That this is connected with the living condition of the cells is evident from the fact that with dead tissue the absorption ratio approximates to unity as shown by Meurer's results already cited, and by the result obtained with dead carrot tissue in 0·02 N sodium chloride by Stiles and Kidd. The absorption ratio found with such dead tissue was 0·88, whereas living tissue under otherwise exactly similar conditions yielded an absorption ratio of 3·5.

This work has been extended by Miss Redfern (1922 b) to the uptake of dyes, the same tissue, carrot root, being for the most part employed. The dyes used were neutral red, methylene blue, methyl violet, aniline blue, eosin and congo red. The absorption ratios found with these dyes presented in various concentrations (initially 0·1, 0·05, 0·01 and 0·005 per cent.) are shown in Table XXXVIII.

TABLE XXXVIII

Absorption ratio at equilibrium in the absorption of various dyes
by disks of storage tissue. (Data from Redfern)

Dye	Character of dye	Concentration at equilibrium		Absorption ratio
		External	Internal	
Neutral red	Basic; semi-colloid	0·06	2·0	33·3
		0·03	1·0	33·3
		0·0005	0·475	950
		0·000125	0·244	1952
Methylene blue	Basic; crystalloid	0·032	3·4	106
		0·01	1·9	190
		0·0008	0·46	575
		0·0003	0·235	783
Methyl violet	Basic; semi-colloid	0·03	3·5	117
		0·01	2·0	200
		0·0018	0·41	228
		0·001	0·20	200
Aniline blue	Basic; colloid	0·1	—	—
		0·045	0·25	5·56
		0·008	0·1	12·5
		0·003	0·1	33·3
Eosin	Acid; crystalloid	0·1	—	—
		0·045	0·25	5·56
		0·0085	0·075	8·82
		0·004	0·05	12·5

A similar influence of concentration of the dye was found with potato immersed in neutral red solutions, with artichoke tuber in solutions of methylene blue and with turnip root in solutions of methyl violet. It will be observed that the absorption ratios are very much higher in the case of basic crystalloidal and semi-colloid dyes than in the case of the acid eosin and the colloidal, although basic, aniline blue, although in both cases the influence of dilution of the dye is to bring about an increase in the absorption ratio. Miss Redfern has shown that the absorption here also represents approximately the relation between concentration and quantity absorbed at equilibrium. Deviations from this rule may be partly due to the . approximate character of determinations by the colorimetric method, and partly to the complicated nature of the absorption process.

The dependence of the position of equilibrium on the nature of the absorbed substance. We have already noted in the case of dyes that the intake of a dye by plant tissue depends very greatly on the nature of the dye. In general basic dyes are absorbed to a very much greater extent than acid dyes, although this rule is not without exception, while among each group of dyes there is considerable range of variation in the extent of intake. Stiles and Kidd (1919 b) have shown the same to be the case with salts. In Table XXXIX are shown the absorption ratios they found in the absorption by carrot tissue from solutions of a number of chlorides, sulphates, nitrates and potassium salts in initially the same equivalent concentration, namely, 0·02 N. The results within each group are strictly comparable.

TABLE XXXIX

Absorption Ratios of a Number of Chlorides, Sulphates, Nitrates and Potassium Salts presented to Carrot Tissue in a Concentration of
0·02 N

Group	Salt	Duration of experiment in hours	Absorption ratio
I	Potassium chloride	91	3·58
	Sodium chloride	,,	3·49
	Lithium chloride	,,	1·16
	Calcium chloride	,,	1·09
II	Potassium sulphate	64·5	0·51
	Sodium sulphate	,,	0·46
	Magnesium sulphate	,,	0·097
III	Potassium nitrate	71·5	4·65
	Sodium nitrate	,,	3·30
	Calcium nitrate	,,	1·19
	Aluminium nitrate	,,	0·53
IV	Potassium chloride	42	1·99
	Potassium sulphate	,,	0·55
	Potassium nitrate	,,	2·20

These results show clearly that the extent to which a salt is absorbed by the particular tissue used depends both on the kation and anion of the salt. Salts having the same anion are absorbed in the order K, Na, Li, [Ca, Mg], Al, while salts containing the same kation (K) are absorbed in the order NO_3, Cl, SO_4. This means, as far as these results go, that salts containing two univalent ions are absorbed much more rapidly than salts containing a divalent or trivalent ion. The difference in the position of equilibrium of K, Na, and Li salts on the one hand, and of Ca on the other, is very striking, and so is the difference in the position of the equilibrium between chlorides and nitrates on the one side and sulphates on the other.

The influence of the thickness of the tissue on the position of equilibrium. The only experiments of which I am aware dealing with this question are those of Ruhland (1909 *b*), who compared the absorption by equal weights of disks of beetroot 3 mm. and 1 mm. in thickness immersed in 0·4 per cent. calcium chloride, and of carrot disks of the same two thicknesses in 1 per cent. ammonium nitrate. His results are shown in Table XL.

TABLE XL

Influence of the Thickness of the Tissue on the Absorption Ratio

Tissue	Salt	Initial concentration in per cent.	Duration of expt. in days	Thickness of tissue in mm.	Absorption ratio	
					Kation	Anion
Carrot	Ammonium nitrate	1	7	3	0·5276	—
,,	,, ,,	,,	,,	1	0·8342	—
Beetroot	Calcium chloride	0·4	2	3	0·2582	0·0354
,,	,, ,,	,,	,,	1	0·3421	0·0522
,,	,, ,,	,,	4	3	0·3266	0·0486
,,	,, ,,	,,	,,	1	0·5616	0·0826

It is clear that in these experiments the extent of absorption by an equal weight of tissue was considerably increased by increasing the surface of the tissue directly exposed to the solution. While this result is understandable on the view that the absorption of the salts examined is controlled by adsorption, yet the results are so few that it would be premature to elaborate an explanation of the results. Clearly the question is deserving of further examination.

THE COURSE OF ABSORPTION OF DISSOLVED SUBSTANCES

The course of absorption of a number of salts by storage tissues (carrot root and potato tuber) at a fixed temperature of 20° C. has been determined by Stiles and Kidd (1919 *a*, *b*) by the electrical conductivity method already described. These investigations yielded

information on (1) the influence of concentration on the rate of intake of salts, (2) the dependence of the rate of absorption on the nature of the salt, and (3) the course of absorption in general. Some information on these questions and on the influence of temperature, light and wounding on the absorption of dissolved substances is also available from other sources.

Influence of concentration. This was investigated for the case of a number of chlorides; namely, those of potassium, sodium and calcium, the salts being used in concentrations of 0·1 N, 0·02 N, 0·002 N and 0·0002 N in each case. The data obtained with each salt are all strictly comparable with each other, the disks of tissue employed being all from one batch.

The actual measurements obtained in the case of carrot are shown for the three salts in Table XLI and the results for potassium chloride are recorded graphically in Fig. 14, where the relative absorption has been assumed proportional to the sum of the fall in conductivity of the solution and the rise in conductivity of the same quantity of distilled water containing the same quantity of the same tissue.

TABLE XLI

Changes in Electrical Conductivity of Solutions of Various Chlorides of Different Concentrations containing Carrot Tissue (40 Disks of Carrot, 1·8 cm. in diameter and 1 mm. thick, immersed in 100 c.c. of each Solution). (Data from Stiles and Kidd)

Salt	Time in hours	Change in conductance of external solution				
		Distilled water	0·0002 N	0·002 N	0·02 N	0·1 N
Potassium chloride	0·5	—	—	− 3	− 167	− 610
	6·0	+ 80	+ 58	− 48	· 372	·· 970
	24·0	+145	+ 92	−196	− 892	··1600
	52·0	+196	⊦137	−223	·· 992	··1850
Sodium chloride	3·0	+ 36	+ 30	+ 18	·· 113	− 560
	34·0	+ 87	+ 57	−124	− 476	−1070
	41·5	+ 66	+ 19	−287	− 885	−1580
	48·0	+ 58	·⊹ 2	−340	−1020	−1720
Calcium chloride	0·5	—	—	+ 3	− 71	− 343
	14·5	+ 64	+ 35	− 53	− 145	− 457
	20·5	+ 86	+ 53	− 57	− 125	− 370
	36·25	+ 60	+ 17	−105	·· 181	− 503
	42·5	+ 54	+ 8	−116	− 195	− 470

It is very clear from these tables and figures that the rate of absorption of salt is dependent on the concentration of the salt, the greater the concentration the more rapid the absorption. Similar results were obtained by Miss Redfern (1922 *b*) with dyes. This does

not mean, of course, that the rate of equilibration is more rapid in the case of stronger solutions, for a greater quantity of salt is absorbed.

The influence of concentration on the absorption of aniline dyes was examined by Szücs (1910). His method is not ideal. Filaments of *Spirogyra* were placed in solutions of methyl violet of different concentrations varying from 0·000125 to 0·00125 per cent. and the time determined that had to elapse for the cells to acquire a standard depth of tint. It was found that the product of time and concentration was a constant over this range of concentrations, from which
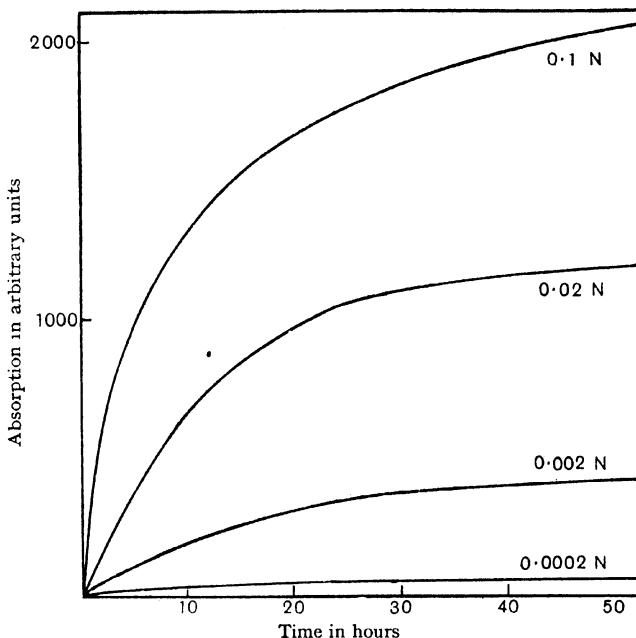
Fig. 14. Absorption of potassium chloride by carrot root tissue immersed in solutions of various concentrations. (From the data of Stiles and Kidd.)

it was concluded that Fick's law holds for the absorption of the dye. It will be remembered that Fick's law states that

$$dQ = -DA \frac{\partial C}{\partial x} dt,$$

where dQ is the quantity of substance diffusing through a cross-section of area A in the time dt, D being the coefficient of diffusion and $\frac{\partial C}{\partial x}$ the concentration gradient in the line of flow. Szücs assumes that

the concentration gradient remains constant in each case throughout the time of the experiment, as the concentration of the external medium remains practically the same, and the dye is bound in the cell in some osmotically inactive form. Hence in the case of each concentration the quantity of dye absorbed will be proportional to the time and hence

$$Q = kCt$$

where Q is the total quantity of dye absorbed in the time t when the concentration of the dye in the external medium is C, and k is a constant depending on the coefficient of diffusion and the dimensions of the cells, presumably assumed equal in all cases.

Since Q is the same in all the experiments, it follows that if Fick's law holds, Ct must be a constant. Experiments with neutral red showed that Fick's law does not hold with regard to the absorption of this dye by *Lemna minor*. This is attributed to a complication arising through the adsorption of this dye by the cell wall. According to Ruhland (1908 *a, b*) this adsorption is prevented by the presence of hydroxyl-ions, and Szücs found that when neutral red was dissolved in 0·005 N sodium hydroxide, the product of the concentration and time required for the absorption of a definite quantity of dye was a constant both when *Lemna* and *Spirogyra* were used.

The influence of concentration on the rate of intake of two alkaloids (piperidine and quinine) and a purine (caffeine) by two species of *Spirogyra*, was examined by Tröndle (1920) by essentially the same method, the time being measured that was necessary for these substances in various concentrations to produce a visible precipitate in the cells. The same result was obtained as Szücs had found in the case of methyl violet, namely, that the product of concentration and time is constant. This result supports the view that Fick's law of diffusion is followed. This is in marked contrast with the results obtained by Tröndle for the intake of salts, to which reference is made later in this chapter.

The rate of absorption of different substances. Four sets of comparative experiments were carried out by Stiles and Kidd to determine the differences in the rate of intake of a number of (1) chlorides, (2) sulphates, (3) nitrates, and (4) potassium salts. All the salts were employed in the same equivalent concentration, namely, 0·02 N, and the experiments were all conducted at the same temperature of 20° C. The relative absorption of the different salts after different times as indicated by the change in electrical conductivity is shown in Table XLII for the nitrates examined.

TABLE XLII.

Absorption of Salt by Carrot Tissue from Solutions of Various Nitrates in a Concentration of o·02 N. (Data from Stiles and Kidd)

Time in hours	Change in conductance of solutions				
	Potassium nitrate	Sodium nitrate	Calcium nitrate	Zinc nitrate	Aluminium nitrate
o·5	− 183	− 89	− 86	− 57	− 20
19·25	− 891	− 659	− 215	+ 107	− 135
71·50	− 2032	− 1360	− 493	+ 540	− 268

These results are in themselves sufficient to show that the initial rate of absorption is dependent on some other factor besides the position of equilibrium. These experiments and those made with chlorides and sulphates indicate that if kations are arranged in order of the initial rate of absorption of salts containing them and a common anion the following series is obtained:

K, [Na, Ca], Li, Mg, Zn, Al,
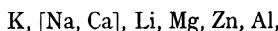
the relative position of ions placed within square brackets being doubtful. The chief difference between this order and that of the total amount of salt absorbed lies in the position of calcium in the series. Mobility of kation and coefficient of diffusion appear to play a considerable part in determining the initial order of absorption, as might indeed be expected.

TABLE XLIII.

Absorption of Salt by Carrot Tissue from Solutions of Various Potassium Salts in a Concentration of o·02 N.
(Data from Stiles and Kidd)

Time in hours	Change in conductance of solutions		
	Potassium chloride	Potassium sulphate	Potassium nitrate
o·25	− 145	− 212	− 197
2·25	− 233	− 258	− 214
19·0	− 550	− 266	− 625
42·0	− 1042	− 331	− 1152

The experimental results with three potassium salts are shown in Table XLIII. From these results it appears that the initial order of absorption of anions is

SO_4, NO_3, Cl,
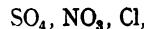
while the final order is NO_3, Cl, SO_4,

the change again being due to the position of the divalent ion, in this case sulphate.

Fitting (1915) and Tröndle (1918 *a*) have attempted to follow the rate of absorption of salt by measuring the rate of deplasmolysis by their methods described in the last chapter. Fitting concluded that potassium chloride and potassium nitrate penetrate with ease the protoplast of the epidermal cells of *Rhœo discolor*, but that potassium sulphate penetrates much more slowly. Sodium chloride and sodium nitrate both penetrate into the cells, whereas lithium chloride and lithium nitrate enter much more slowly. Magnesium chloride, nitrate, and sulphate only penetrate slowly, while no penetration of calcium and barium salts examined could be observed. The order of absorption found by Fitting for kations was thus

[K, Na], Li, Mg, [Ca, Ba],

and for anions [NO_3, Cl], SO_4.

Later, Fitting (1919) examined the permeability of the same and other cells to glycerol and urea and concluded that urea was absorbed at about the same rate as potassium nitrate or sodium chloride, but that glycerol was absorbed much more rapidly.

Tröndle employed the same method in order to determine the intake of salts by roots of *Lupinus albus* and palisade cells of the leaves of *Acer platanoides* and *Salix babylonica*, and decided that kations were absorbed in the order

Rb, K, Li, Mg, Ba, Sr, Ca,

and anions in the order NO_3, Cl, SO_4.

Kahho (1921 *d*) investigated the entrance of a number of salts into the young roots of the yellow lupin by means of Lundegårdh's method of tissue contraction and extension described in the last chapter. In order to obtain comparable results with different salts isotonic solutions were used, Fitting's values (1917) of isotonic coefficients being accepted. Kahho came to the conclusion that the order of absorption of kations is

K, Na, Li, Mg, [Ca, Ba],

while the order for anions is

[Br, I, NO_3], Cl, tartrate, SO_4, citrate.
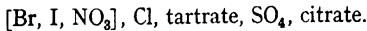
While all these results agree in the main with those of Stiles and Kidd, it is clear from the results obtained by the latter workers that the plasmolytic and tissue extension methods cannot be expected to give a quantitative measure of absorption. For these latter methods are based on the assumption that after entering the cell the absorbed substance remains in solution in the same condition

as it previously existed outside, so that if it were capable of entering the cell it would do so until there were equality of concentration inside, and outside the cell, unless the permeability of the cell membranes to the substance became reduced to approximately zero. But as a matter of fact this assumption cannot be correct, for the position of equilibrium depends on the nature and concentration of the substance. Consequently the rate of deplasmolysis does not necessarily give a measure of the total absorption of solute, but only of the increase in the osmotic concentration of the cell sap, which may be a very different thing if the whole of the absorbed solute does not remain in solution and so retain its osmotic activity.

That such a complication is possible was indeed recognised by Fitting (1919)[1], and also by Höfler and Steigler (1921) who investigated the intake of urea by means of Höfler's plasmometric method. Some of the results obtained by Höfler and Steigler may be quoted. They found a tissue, the red-violet epidermis of the stem of *Gentiana Sturmiana*, which absorbs urea with remarkable rapidity. The mean osmotic concentration of these cells lies between 0·4 M and 0·55 M sucrose. When these cells, after a preliminary washing in water for about twenty minutes, are placed in a gram-molecular solution of urea, the intake of urea is such that the concentration of this substance inside the cells increases by about 0·02 to 0·07 gram-molecular per *minute*. This compares with about 0·05 and 0·06 gram-molecular per day found for the intake of this substance by the epidermal cells of the under side of the midrib of the leaf of *Rhœo discolor* by de Vries (1889 b); 0·008 to 0·016 gram-molecular per hour found for the same tissue by Fitting (1919); 0·01 to 0·03 gram-molecular per hour by the parenchymatous cells of the internodes of *Tradescantia elongata* immersed in a plasmolysing solution of concentration 0·50 M found by Höfler; and 0·04 to 0·11 gram-molecular per hour found by the same author for the intake of urea by the bulb scales of *Allium Cepa*. If the concentration gradient is taken into account, the absorption of urea by the epidermal cells of the stem of *Gentiana Sturmiana* is thirty times as rapid as in the bulb scales of *Allium Cepa*, forty-five times as rapid as in the case of *Rhœo discolor* and sixty times as rapid as in the case of *Tradescantia elongata*.

This rapid intake is not shown by other substances, for the same cells of *Gentiana Sturmiana* immersed in 0·60 M potassium

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[1] And by S. Prát ("Plasmolyse und Permeabilität," *Biochem. Zeitsch.*, **128**, 557–567, 1922), who, using the plasmometric method, obtained results with *Spirogyra* and various salts agreeing closely with those summarised above.

nitrate absorb this salt so that (in the mean) the concentration inside the cells increases at the rate of about 0·006 gram-molecular per hour. This is of the same order as the rate found by Fitting and Höfler for *Rhœo discolor* and *Tradescantia elongata* respectively. Thus, we have evidence that the order of intake of different substances is different with different tissues, for in the case of epidermal cells of *Gentiana Sturmiana* urea appears to be absorbed about 170 times as fast as potassium nitrate, but in the epidermal cells of the leaf of *Rhœo discolor* at about the same rate, while Höfler finds for the stem parenchyma cells of *Tradescantia elongata* that potassium nitrate may be absorbed up to five times as fast as urea.

It is also interesting that other cells of *Gentiana Sturmiana* do not show the same high rate of absorption[1] of urea, as the sub-epidermal cortical cells were found to absorb this substance so that its concentration inside the cells increases at the rate of about 0·002 gram-molecules per minute. A similar difference between epidermal and cortical cells was also observed in *Euphrasia Rostkoviana, Melampyrum sylvaticum, Veronica Beccabunga, Homogyne alpina* and *Taraxacum officinale.*

A high rate of uptake of urea, as well as of ethyl alcohol, anti-pyrin and oxalic acid, by the curious hairs extruded from the epidermal cells of the seeds of *Cuphea lanceolata* immersed in water, has been recorded by van Wisselingh (1920), but it is doubtful whether van Wisselingh's experiments were rightly interpreted, for it is not at all certain whether we are here concerned with an action of the living cell or with reactions of a dead constituent of the epidermal cells (cf. Ruhland, 1922).

Van Wisselingh concluded further that his observations indicated that the hairs in question are less permeable to glycerol and tartaric acid than to urea and the other substances mentioned above, while sucrose is not absorbed at all rapidly.

Little information is available with regard to the influence of the nature of the substance on the course of absorption of other organic substances by plant tissue. In the case of dyes, the absorption of which has been such a favourite object of observation, chiefly equili-brium conditions, or conditions approaching those of equilibrium, have been investigated. Miss Redfern (1922 *b*) has, however, followed the course of absorption of neutral red, methylene blue and methyl violet by carrot tissue, and comes to the conclusion that curves

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[1] Höfler uses the term permeability as practically synonymous with rate of absorption.

obtained representing the relation between absorption and time are similar to those obtained by Stiles and Kidd for the intake of salts by the same tissue, absorption being rapid at first, but slowing down considerably after the first few hours of absorption and then proceeding slowly towards a position of equilibrium. In other cases where only a little dye is absorbed at equilibrium, as with aniline blue and eosin, equilibrium appears to be reached very rapidly; so rapidly that the data necessary for constructing time-absorption curves could not be obtained.

With dead tissue the initial rate of absorption of methylene blue and other dyes that accumulate in the cells was found to be very much slower than in the case of living tissue, and considerably less dye is absorbed at equilibrium; while the difference between the high initial rate of intake and the later slow rate of absorption is very much less marked.

Influence of temperature on the rate of absorption. The effect of temperature on the rate of absorption was examined by Stiles and
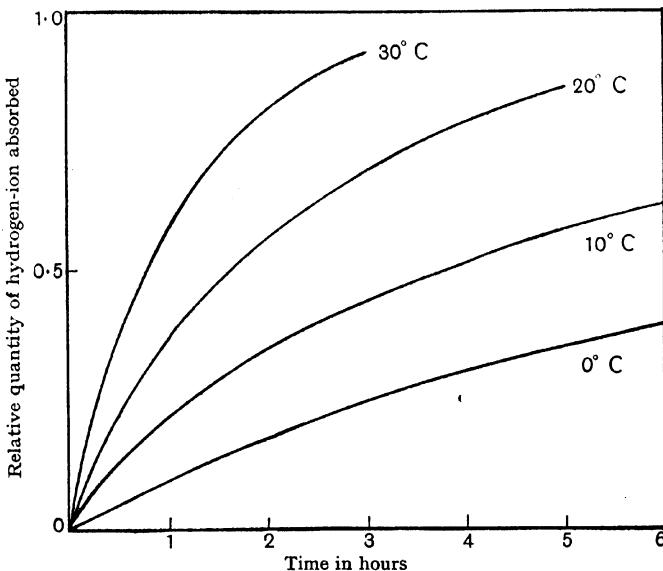
Fig. 15. Curves to illustrate the absorption of hydrogen-ion by potato tuber tissue immersed in 0·0011 N hydrochloric acid at various temperatures. (From the data of Stiles and Jørgensen.)

Jørgensen (1915 *b*) for the case of the absorption of hydrochloric acid, or rather the hydrogen ions of that acid, by potato tuber. The tissue

was used in the form of circular disks 1·0 cm. in diameter and 0·2 cm. in thickness, 20 disks being immersed in 100 c.c. of the acid solution. The latter was used in a concentration of 0·0011 N and changes in its value with time were determined electrometrically by means of the hydrogen electrode. The absorption of the acid was followed in this way at temperatures of 0°, 10°, 20° and 30° C. The relation between time and absorption found in these experiments is represented graphically in Fig. 15. The relation is almost a logarithmic one, so that the equation representing the rate of absorption is approximately

$$\frac{dx}{dt} = k\,(A - x),$$

where $\frac{dx}{dt}$ is the rate of absorption at any time when x is the diminution in the concentration of acid in the external solution and A represents the initial concentration. In this equation k is determined by the temperature and in Table XLIV are shown values of k calculated for the different temperatures employed. From these results it appears that the rate of absorption of hydrogen ions by potato tuber is increased about 2·2 times for every rise of temperature of 10° C. within the temperature range 0° C. to 30° C.

TABLE XLIV

Influence of Temperature on the Rate of Absorption of Hydrogen Ions by Potato Tuber. (Data from Stiles and Jørgensen)

Temperature in centigrade degrees	"k"
0	0·036
10	0·081
20	0·174
30	0·380

This temperature coefficient (Q_{10}) is that characteristic of chemical reactions rather than of physical reactions such as diffusion or adsorption. But having regard to the great variations in the value of the temperature coefficient in different cases of the absorption of water by plant tissue, it would clearly be unwise to draw far-reaching conclusions with regard to the nature of the process of absorption of hydrogen ions from the temperature coefficient alone. But from the magnitude of the absorption it seems clear that something more than simple diffusion alone is required to account for the process. Thus, after three hours in the acid at 30° C. the potato tissue had absorbed so much acid that the absorption ratio was 39 and there was no indication that equilibrium had been reached.

Whether chemical action or absorption is mainly responsible for this result cannot at present be said definitely any more than it can be for the case of salts.

A few observations are on record indicating that the rate of intake of dyes is increased by rise in temperature. Pfeffer (1886) found this the case with methylene blue and Endler (1912 *b*) for neutral red. Collander (1921) has recently made some observations on the influence of temperature on the intake of some sulphonic acid dyes (orange *G* and cyanol), and concludes that temperature has a considerable effect on the intake of these dyes by the pith cells of *Tropæolum*. Similar results were obtained with the parenchyma cells of perianth leaves of *Hyacinthus*, although the temperature effect was not so great. As, however, the intake of dye was not followed with time, information relative to the temperature coefficient is not forthcoming.

Influence of light on the rate of absorption. The influence of light on the rate of absorption of sodium chloride by palisade cells of *Acer platanoides*, *Salix babylonica* and *Buxus sempervirens* was examined by Tröndle (1918 *b*) by the deplasmolytic method. The results indicate that increasing illumination increases the rate at which the salt passes into the cells up to a maximum with a certain light intensity. This intensity varies with the different cells examined; with *Acer platanoides* it was about 1250 metre candles, with *Salix babylonica* about 9375 metre candles and with *Buxus sempervirens* about 1500 metre candles. Results obtained previously by the same author (1910) by the method of permeability coefficients gave similar indications[1]. On the other hand, Ruhland (1911), using this latter method, found that light was without measurable influence on the permeability of the cells of the sugar beet leaf to sucrose, glucose and fructose.

Using this same method Lepeschkin (1909 *b*) showed that the cells of the pulvini were more rapidly penetrated by dissolved substances after illumination than in the dark, an observation confirmed by Blackman and Paine (1918) by determining the exosmosis of electrolytes by means of changes in the electrical conductivity of the external liquid. For reasons already given, neither the method of permeability coefficients nor the deplasmolytic method can be regarded

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[1] D. R. Hoagland and A. R. Davis ("Further Experiments on the Absorption of Ions by Plants, including Observations on the Effect of Light," *Journ. Gen. Physiol.* **6**, 47–62, 1923) have recently shown that a number of ions are more rapidly absorbed by *Nitella* in the light than in the dark. They consider that light is important here as a source of energy required for the absorption of ions from dilute solutions rather than that permeability is directly influenced by light.

as capable of yielding exact quantitative data with regard to the passage of substances into the cell. However, a more rapid rate of deplasmolysis can ʻprobably be accepted as indicating more rapid entrance of dissolved substance, and a higher permeability coefficient as indicating the same thing.

Kahho (1921 *b*) found that upper epidermal cells of the leaf of the red cabbage placed in a 0·48 *N* solution of sodium iodide at a temperature of 23-24° C. were killed more rapidly in light than in the dark. Similar results were obtained with sodium bromide and sodium thiocyanate. These results were attributed by Kahho to a more rapid entrance of the salts in question in the light than in the dark.

Influence of the thickness of the tissue on the rate of absorption. Höfler and Stiegler (1921), using the plasmometric method, observed that urea entered the epidermal cells of the stem of *Gentiana Sturmiana* much more slowly in very thin sections which contained only the epidermal layer, but this uninjured, than in thick sections. The explanation of this is not forthcoming; it might, perhaps, be correlated with the observations of Tröndle (1921) on the effect of wounding on permeability to which reference is made in the next chapter.

On the course of absorption in general. The curves obtained by Stiles and Kidd for the course of absorption of salts show that there is a rapid intake of salt for the first 15 or 30 minutes after which absorption follows an approximately logarithmic course towards the position of equilibrium. How exactly the latter part of the curve, or the whole of it, is represented by a logarithmic equation, cannot be said; in the case of the absorption of hydrogen ions by potato tuber for a few hours the approximation was near enough for the assumption of this relation in the calculation of the influence of temperature.

As the position of equilibrium differs in the case of every substance and of every concentration of the same substance, at any rate as far as existing data indicate, it is perfectly clear that the rate of absorption cannot be regarded as a measure of the permeability of cell membranes to the substance. For if the permeability of the cell membranes to potassium chloride and calcium chloride were the same, the rate at which the calcium salt entered the cell would soon slow down as compared with the rate at which the potassium salt entered, on account of the different position of the equilibrium.

The explanation given by Fitting (1915) of the slowing down of the rate of deplasmolysis is that the salt itself lowers the permeability of the cell to the salt. That this explanation is inadequate becomes

clear from the fact that the position of equilibrium bears a definite relation to the concentration of the external solution, and that in low concentrations the absorption ratio may be ·many times unity, a result which cannot be explained on the ground of changes in permeability of the membrane alone. As the concentrations used by Fitting are 'very high, it is to be expected that the position of equilibrium will be attained when the absorption ratio has reached a position considerably below unity.

Tröndle considered his results as indicating that there was a rapid intake of salt during the first 10 minutes of experiment, during which time the intake of salt was proportional to the time of action and independent of the concentration, after which the rate of intake gradually lessened according to a logarithmic relation. That Tröndle's method is capable of giving such accurate data I very much doubt for reasons already stated. Tröndle's theoretical conclusions from these results are somewhat surprising; they will be mentioned on a later page.

Höfler (1918 *b*, 1919) investigated the absorption of potassium nitrate by cells of *Tradescantia elongata* and *Rhœo discolor* by means of the plasmometric method described in the last chapter. This method, it will be recalled, attempts to measure the intake of salts by individual cells. Höfler found extraordinarily wide variations in the rate of absorption of the salt by different cells of the same tissue. Thus in a section of parenchymatous tissue of *Tradescantia elongata* the quantity of salt absorbed by individual cells in the same time varied between 0·009 gm. mol. and 0·043 gm. mol. with a mean value of 0·022 gm. mol. Moreover, the quantity of salt apparently absorbed by the same cell may undergo as wide variations with time. These changes in rate of intake appear quite independent of the abnormally high rate of intake which is an indication of the death of the cell. A general decrease in the rate of intake as observed by Fitting was not observed by Höfler until the lapse of about a day or longer from the first immersion of the tissue in the solution.

The course of absorption of the two ions of a number of salts by whole plants of a considerable number of different species was investigated by Pantanelli (1918) by analysing the external solutions for both ions. He came to the conclusion that the two ions are absorbed independently throughout the whole course of absorption. The rate of absorption varied greatly with different ions and different salts, and ions that were absorbed most rapidly by one species were not necessarily absorbed with any great rapidity by another species. Generally speaking, other factors being equal, unicellular organisms

or those rich in protoplasm, absorb ions much more rapidly than multicellular organisms or those poor in protoplasm.

Pantanelli found that in some cases oscillations occurred in the total quantity of ion taken in by certain of the plants he examined, indicating alternating periods of absorption and excretion. This was observed for example in *Vicia Faba* in potassium bromide, sodium monohydrogen phosphate, calcium nitrate and barium nitrate; *Valonia utricularis* in ammonium sulphate, magnesium sulphate, potassium dihydrogen phosphate, ammonium nitrate or potassium bromide; yeast in zinc sulphate or calcium chloride. But as the method of Pantanelli involves the use of different plant material for each analysis, it seems possible that the differences recorded resulted from differences in the absorbing capacity of the various batches of plants used. Pantanelli is aware of this difficulty, but thinks that as a source of error it is negligible because he had four plants in every vessel and took care to use plants having equal development both of root and shoot.

I think it is likely Pantanelli has underrated the magnitude of variation possible among plants that appear very similar to the eye. Miss Redfern (1922 *a*) performed similar experiments on the absorption of calcium and chlorine ions from solutions of calcium chloride by living plants of *Pisum sativum* and *Zea Mays*. Careful selection of plants that appeared equally developed was made at the commencement of the experiment, each plant was grown singly in water culture and for any determination of absorption separate analyses were made of the solutions in which six plants had been growing. From these results it was possible to calculate the probable error of the determination of absorption, and although owing to the small number of plants no great exactitude could be claimed for the results, yet these brought out very plainly the great variations that may occur in absorption by apparently exactly similar plants. The results also showed that within the limits of probable error there was no indication of such a periodicity in absorption as that recorded in a number of cases by Pantanelli, absorption proceeding continuously to an equilibrium value attained in the case of peas after about 24 hours, and in the case of maize after about 48 hours. Subsequent excretion of the salt appears to be due to the approach of death of the plant. Further work with other plants is necessary, however, before it can be denied with certainty that the apparent oscillations observed by Pantanelli can be definitely explained as due to differences between individual plants.

THE INFLUENCE OF THE PRESENCE OF ONE DISSOLVED
SUBSTANCE ON THE ABSORPTION OF ANOTHER

So far, our consideration of the intake of dissolved substances has been limited to substances presented to the plant in pure solution. We have now to consider how this intake is affected when more than one substance is present in solution in the external medium. It has already been mentioned in Chapter IV that the presence of a non-electrolyte like sugar or glycerol reduces the rate of diffusion of an electrolyte, while in Chapter V reference has been made to the retardation or inhibition of diffusion through porcelain membranes that can be brought about by blocking the pores of the membrane with some substance. It would not then be surprising to find that the presence of one substance in the external solution should hinder the intake of another.

That the presence of a second substance dissolved in the external medium reduces the harmful action of a toxic substance in the solution external to living tissue has long been recognised (cf. Brenchley, 1914 *b*, Stiles and Jørgensen, 1914 *b*). Such a result does not, in itself, prove that the actual entrance of the toxic substance is prevented or retarded. The seat of the depoisoning action may be inside the plant. However, there is considerable evidence that this "antagonism" between dissolved substances is actually due to a mutual hindrance to their entrance into plant cells; this evidence it will be necessary for us to discuss.

This antagonistic action was brought into prominence by the work of Loeb (1900, 1901, 1902, 1905, 1906; Loeb and Gies, 1902) on the development of the eggs of the marine fish *Fundulus*. Development of these eggs is inhibited if they are put immediately after fertilisation into a pure solution of sodium chloride having the same concentration as that of this salt in sea water. But if a small quantity of the salt of a bivalent metal such as calcium, strontium, magnesium, or even lead, is added to the sodium chloride solution, development of the egg into an embryo is able to proceed. The result depends on the kations, as different salts of the same metals are able to act equally well as depoisoners. The antagonism between monovalent and bivalent kations is also shown in the depoisoning action of a monovalent kation towards the poisonous action of a bivalent metal such as zinc. Slight antagonism was also observed between two divalent kations, as, for example, strontium and magnesium.

With regard to quantitative relations between the two ions, the quantity of the depoisoning ion required to inhibit the action of the poisonous ion varies with the concentration of the latter. Thus a concentration of 0·25 *N* NaCl is harmless to *Fundulus* eggs; with a concentration of 0·625 *N* NaCl one bivalent ion is required to render 1000 sodium ions harmless. Between these two concentrations the number of bivalent ions relative to the number of sodium ions is less the lower the concentration of the sodium chloride, while above a certain concentration of sodium chloride it is impossible to inhibit the toxic action of the sodium chloride.

These antagonistic effects only appear so long as the fish is surrounded by the egg membrane. Loeb concludes that the membrane is the seat of the antagonistic action, and that it is here that the ions mutually hinder one another in penetrating into the egg.

Loeb's work on antagonism in animals was followed up in plants by Osterhout, who devised a number of methods of attacking this question. In his earliest experiments antagonism in plants was examined by the reduction of toxic action in mixed solutions as compared with the toxicity of pure solutions containing the same substances in the same concentration. The first experiments were with marine plants (Osterhout, 1906 *a, b, c*). It was found that while some marine plants are quickly killed when placed in distilled water, others can live a long time in this medium[1]. The latter plants are, however, killed much more quickly when placed in a pure solution of sodium chloride isotonic with sea water. The toxic action of the sodium chloride is removed to a very great extent by the addition of a little calcium chloride, for in a mixture containing 1 c.c. of 3/8 *M* calcium chloride + 100 c.c. 3/8 *M* sodium chloride, the plants live nearly as long as in distilled water. If some potassium chloride is added to the mixture the plants live longer than they do in distilled water, while with further addition of magnesium chloride and magnesium sulphate, the plants live practically as long as in sea water.

The toxicity of sodium chloride is also reduced by the addition of potassium chloride or magnesium chloride, but not so much as by the addition of calcium chloride. The addition of potassium chloride + calcium chloride reduces toxicity more than magnesium chloride + calcium chloride, and this mixture more than magnesium chloride + potassium chloride.

[1] Possibly owing to exosmosis being less in the case of the latter (cf. Osterhout, 1917 *b*).

Similar results were obtained by Osterhout (1907) with a number of freshwater and terrestrial species including *Spirogyra, Vaucheria, Lunularia* gemmæ and *Equisetum* spores, as well, as wheat seedlings and other flowering plants. In this and subsequent investigations, Osterhout (1907, 1908 *c*) introduced a second method of investigating antagonism, namely, by determination of differences in growth rate. Wheat seedlings, for example, were grown with their roots in the experimental solutions, and the amount of growth determined by measuring the increase in length of the roots in the various solutions after a definite time. Various chlorides and nitrates were employed. In this way it was shown that root development proceeded more rapidly in solutions containing both salts of sodium and potassium, sodium and ammonium, sodium and calcium, and sodium and magnesium, than in pure solutions of the same concentration (0·12 *M*). For each pair of salts there is a definite ratio of the two constituents in which the rate of growth is a maximum.

In a separate investigation (1908 *a*) Osterhout showed that the toxicity of magnesium salts (chloride, sulphate and nitrate) was reduced by the addition of the corresponding potassium salt. Not only is the toxicity of the magnesium reduced by the potassium, but the toxicity of potassium is reduced by the presence of a magnesium salt, for a solution containing both the potassium and magnesium salt is less toxic than a pure solution of either salt in the same concentration.

Much the same method was used by Hansteen (1910) who employed a Norwegian variety of wheat, seedlings of which were maintained for 14 days with their roots in the experimental solutions and the root development then estimated by determining the dry weights of the roots. Nitrates were used in concentrations considerably lower than those employed by Osterhout. By this method a definite antagonism was observed between potassium and calcium, a rather slight antagonism between potassium and magnesium, and a very little, scarcely noticeable, antagonism between potassium and sodium. Antagonism was observed in solutions having a concentration as low as 0·00125 *N*.

Further work on these lines was carried out by McCool (1913) who worked with wheat and Canadian field peas allowed to grow in the experimental solutions for 21 to 30 days. Growth was estimated by measurement of length and determination of dry weights of roots and shoots. Antagonism between calcium and the following metallic ions was found: magnesium, potassium, sodium, ammonium, barium

and strontium. Antagonism was also found between barium and potassium, barium and magnesium, and between strontium and the following: potassium, sodium and magnesium. McCool also observed antagonism between sodium and potassium and between sodium and ammonium. The poisonous action of manganese is reduced by the presence of calcium, potassium, sodium or magnesium.

Antagonism can also be demonstrated by means of determinations of the electrical conductivity of living tissue. It has been mentioned earlier that dead tissue possesses a much greater electrical conductivity than living tissue. Osterhout (1912 *a*, 1914 *f*, 1915 *b*) showed that tissue of *Laminaria* lost in electrical resistance much more rapidly in a solution of sodium chloride having the same conductivity as sea water than in a solution of pure calcium chloride of the same conductivity, while in a solution of this same conductivity containing sodium chloride and calcium chloride in the proportion of 1000 molecules of the former to 15 of the latter, *Laminaria* maintained its original resistance for 24 hours, indicating that in the mixed solution the cells of the alga remain alive considerably longer than in pure solutions of the same conductivity. By this method antagonism has also been demonstrated between sodium chloride and magnesium chloride, sodium chloride and hydrogen chloride (Osterhout, 1914 *i*, 1915 *a*), sodium chloride and sodium taurocholate (Osterhout, 1919 *b*) and between sodium chloride and a purine, caffeine, and an alkaloid, cevadine sulphate (Osterhout, 1919 *d*). By the same method antagonism between sodium acetate and sodium sulphate has been shown by Raber (1920 *b*), but between sodium citrate and a number of other sodium salts (chloride, sulphate, nitrate, iodide and thiocyanate) the reverse effect, an increasing of toxicity, for which the name synergy is suggested, has been observed (Raber, 1917).

Brenner (1920) considers that he has demonstrated the existence of antagonism between hydrochloric acid and a number of salts by the following experiments. He had earlier (1918) examined the resistance of hypodermal cells of red cabbage to acids and alkalies in low concentrations, using plasmolysis and deplasmolysis as tests of the vitality of the cells examined. Pieces of tissue were placed in a solution of 20 per cent. sucrose containing the acid in definite concentration and left in it for the experimental time (ten minutes to four hours). The pieces of tissue were then transferred to 10 per cent. sucrose, then into 5 per cent. sucrose for 30 minutes and, finally, into distilled water for the same length of time. If the cells deplasmolysed normally they were considered to be living. If dead,

the protoplast was either disorganised or was in a fixed plasmolysed condition. In this way the critical concentration of a number of acids, that is, the concentration necessary to injure the cells in a certain time, was found for different plant cells. The critical concentration of hydrochloric acid, for example, in the case of hypodermal cells of red cabbage immersed for four hours in the sucrose solution containing the acid, was $N/700$, corresponding to a hydrogen-ion concentration of $1\cdot4 \times 10^{-3}$.

In his experiments on antagonism a similar procedure was employed, the hydrochloric acid in various concentrations being added to a number of salts in solutions which gave approximately equal plasmolysis. The sections were placed for 20 minutes in these plasmolysing solutions, then for four hours in the solution of salt + acid. They were then transferred successively into a half-strength salt solution, a quarter-strength salt solution and distilled water. The concentrations of salt used, the concentration of hydrochloric acid in the salt solution, and the actual hydrogen-ion concentration, determined by the hydrogen electrode, of the mixed solution, are shown in Table XLV.

TABLE XLV

Critical Concentration of hydrochloric acid in regard to hypodermal cells of red cabbage in presence of various salts and sugars.
(Data from Brenner)

Salt or sugar	Concentration of salt or sugar in per cent.	Critical concentration of hydrochloric acid	Actual hydrogen-ion concentration of the solution
Sodium chloride	2·2	1×10^{-3}	$8\cdot91 \times 10^{-4}$
Potassium nitrate	3·75	$1\cdot25 \times 10^{-3}$	$1\cdot29 \times 10^{-3}$
,, chloride	2·8	$1\cdot67 \times 10^{-3}$	$1\cdot38 \times 10^{-3}$
,, sulphate	5·0	$2\cdot5 \times 10^{-3}$	$4\cdot68 \times 10^{-4}$
Magnesium nitrate	8·8	1×10^{-3}	$1\cdot09 \times 10^{-3}$
,, chloride	7·0	$2\cdot5 \times 10^{-3}$	$3\cdot16 \times 10^{-3}$
,, sulphate	16·1	4×10^{-3}	$1\cdot12 \times 10^{-3}$
Calcium nitrate	6·5	2×10^{-3}	$1\cdot95 \times 10^{-3}$
,, chloride	6·2	4×10^{-3}	$5\cdot50 \times 10^{-3}$
Glucose	—	$1\cdot43 \times 10^{-3}$	$8\cdot90 \times 10^{-4}$
Sucrose	20	$1\cdot43 \times 10^{-3}$	$8\cdot71 \times 10^{-4}$

It will be observed from the data presented in this table that the different substances examined affect very differently the resistance of the cells to hydrochloric acid. Sodium chloride has the same effect as sugar, while potassium sulphate lessens the resistance of the cells to about half. All the other salts, on the other hand, raise considerably the resistance of the cells to acids, as a much higher hydrogen ion concentration is necessary to injure the cells in the presence of the salts.

From these and other observations, as, for example, on the inhibition of the toxic action of salts of copper and heavy metals by salts of the alkali and alkaline earth metals in regard to the germination of fungus spores (J. F. Clark, 1901, 1902; Hawkins, 1913) there can be no doubt that antagonism is a very general phenomenon. But from these investigations, in which antagonism is determined by time taken to kill, amount of growth, or germinative capacity, there is no direct evidence that the antagonistic action of the substances concerned is due to a mutual hindrance to the absorption of the two substances. The antagonism may result from reactions in the external solution, or in the cells themselves after the entrance of the substances. It is clear that the former can scarcely be a general explanation, as antagonism has been observed to take place between so many substances which do not react with one another. Evidence that the antagonism is indeed due, at any rate in some cases, to a mutual hindrance to the entrance of substances into the cell, has been forthcoming from other lines of experimentation.

An observation of Benecke (1907), which appears to have been somewhat overlooked, affords more direct evidence of the effect of one electrolyte on hindering the entrance of another. As is well known, the entrance of an iron salt into the cells of a species of *Spirogyra* containing tannin, is rendered evident by the formation of a green or blue compound. Benecke observed that the entrance of ferrous sulphate into such cells is much delayed by the addition of a calcium salt to the solution of ferrous sulphate. This observation was confirmed by Szücs (1910), who also found that the same result could be brought about by a number of salts, the antagonising action being a function of the valency of the kation, the higher the valency the greater the antagonising action.

These results appear, at first sight, to be in complete discordance with those of Miss Williams (1918 *b*) who found that cells which did not normally absorb ferric chloride would do so after treatment with solutions of various nitrates. It will be observed, however, that in Benecke's experiment the two antagonising salts were present together in the same solution, whereas in Miss Williams' experiments they were present singly and presented to the tissue one after the other.

The non-entrance of the toxic substance in the reduction of toxic action by addition of some other substance to the toxic solution is also indicated by observations on the effect of barium salts on cells of *Spirogyra*. Osterhout (1916 *e*) showed that the chloroplasts of this

alga underwent a peculiar and characteristic contraction in solutions of barium chloride as dilute as 0·0001 M. Strontium chloride produced the same contraction in concentrations of 0·001 M and upwards. Chien (1917) found the same contraction could be produced with cerium chloride in a concentration of 0·00005 M, in a large species of *Spirogyra*, but not in a small one. In the smaller species it was found that the effect of barium chloride was inhibited by addition of calcium chloride or cerium chloride to the solution in the correct proportions, but that no antagonism could be observed between barium and strontium.

A plasmolytic method has been used by Osterhout (1911, 1912 c, 1913 b). It was found that cells of a species of *Spirogyra* were just plasmolysed in 0·2 M calcium chloride and in 0·38 M sodium chloride, but were not plasmolysed in 0·195 M calcium chloride nor in 0·375 M sodium chloride. However, the solution obtained on mixing 10 volumes of the 0·375 M sodium chloride solution with 1 volume of the 0·195 M calcium chloride solution at once brought about plasmolysis. The failure of the solutions of the single salts in these concentrations to bring about plasmolysis is held to indicate the penetration of the solute into the cells in these cases, while the occurrence of plasmolysis with the mixed solution indicates the non-entrance of the solutes from the mixed solution. As the osmotic concentration of the mixed solution is so little different from (actually a little less than) that of the pure sodium chloride solution, and as the concentration of sodium chloride is reduced by only about 9 per cent., it seems improbable that the smaller rate of entrance of the salt on account of the lower concentration of sodium chloride would alone be sufficient to account for plasmolysis taking place in the mixed solution, and it seems most likely that the capacity for the molecules of sodium chloride to enter the cell is reduced.

The influence of one salt on the intake of another by young roots of yellow lupin has been investigated by Kahho (1921 d) by the tissue extension method. It will be recalled that Kahho found kations absorbed in the order K, Na, Li, Mg, [Ba, Ca], while anions fall into the series [Br, I, NO_3], Cl, tartrate, SO_4, citrate. When the entrance of single salts and that of mixtures of two salts are compared, the solutions in all cases being isotonic, it is found that the entrance of any kation in the series is retarded by the presence of any other kation to the right of it in the series, the further the kation lies to the right the greater the retarding effect. Thus, potassium chloride enters much more rapidly than lithium chloride presented in the

same osmotic concentration, but from a mixture of the two having the same total osmotic concentration (0·15 KCl + 0·048 M LiCl), salt enters a little faster than from the pure lithium chloride solution (0·192 M) but much more slowly than from the pure potassium chloride solution (0·200 M).

The same rule was found to hold for anions. The action of any salt in furthering or retarding intake was found to be the sum of the action of its two ions.

While it is clear from earlier considerations put forward in this chapter dealing with the unequal absorption of ions, that the plasmolytic test is not free from objection, direct evidence that antagonistic action is due to mutual hindrance to intake is forthcoming from chemical analysis of tissues and colorimetric determinations in cells and external solutions. By such means it has been shown by Szücs (1911, 1912) that roots of *Cucurbita Pepo* rapidly absorb enough copper from pure solutions of copper sulphate not only to inhibit the response of the hypocotyl and root to gravity and light, but also to give a very definite qualitative test for copper. If aluminium chloride is added to the solution of copper sulphate, the inhibition of the geotropic and phototropic reaction is delayed and the copper is absorbed to a much less extent in the same time. The maximum depoisoning effect is produced with a ratio of 0·15 N aluminium chloride to 0·025 N copper sulphate.

From water culture experiments with *Eriophorum vaginatum*, *Phragmites communis* and *Carex riparia*, Stoklasa, Šebor, Týmich and Cwacha (1922) conclude that they find antagonism between aluminium and ferric ions. These plants were maintained with their roots in complete nutrient solutions which in some cases contained in addition aluminium nitrate, in others ferric nitrate and in yet others both these salts. By analysis of the experimental solutions after the lapse of 13 days it was found that less iron and aluminium were absorbed from the solution containing both these salts than from those containing only one, although the conditions were otherwise equal and the concentration of both the aluminium nitrate and the ferric nitrate was the same, namely 0·002 N, as in the solutions containing only one of these salts. Thus, in the experiments with *Phragmites communis* the quantity of aluminium absorbed from the solution containing this without iron was 51·3 mgm., the quantity of iron absorbed from the solution containing this without aluminium was 46·6 mgm. while from the solution containing both, 27·3 mgm. aluminium and 8·4 mgm. iron were absorbed.

By direct tests of the cell sap of *Nitella*, Miss Brooks (1922) has shown that lithium, calcium and strontium ions enter the cells of this plant less rapidly from mixed balanced solutions than when presented singly. From determinations of the electrical conductivity of the sap of the same species Osterhout (1922 *b*) concludes that the nitrate ion rapidly penetrates the cells when sodium nitrate is presented to the plant in pure solution, whereas when the sodium nitrate is balanced by the addition of calcium nitrate the rate of entrance is much slower.

So far we have considered only the antagonism between inorganic salts, or, more correctly, between ions, for, as we have already seen, the ions of a salt may be absorbed to different extents. Antagonism has also been observed between inorganic salts and organic substances such as dyes and alkaloids. Thus, von Eisler and von Portheim (1909) found the toxic action of quinine reduced by the presence of salts of sodium, calcium, magnesium and aluminium.

Szücs (1912) examined particularly the antagonism between quinine hydrochloride and potassium nitrate, calcium nitrate and aluminium nitrate in the case of *Spirogyra*. The absorption of the quinine is made visible by the precipitation of tannin in the cells, while as a test of vitality, centrifuging the filaments was employed, the plasma and chloroplasts remaining fixed if the cells are dead, but not otherwise. Each of the three salts used was able to antagonise the toxicity and entrance of the quinine, the depoisoning effect increasing with the valency.

That antagonism is actually due to the non-entrance of the toxic substances is indicated by the observation of Szücs on the toxic action of piperidine in presence and absence of various inorganic salts. For some unknown reason the absorption of piperidine is not hindered by inorganic salts, and the toxicity of the piperidine is increased rather than diminished by the addition of an electrolyte.

Following up the work of Szücs on the antagonistic action of aluminium nitrate and quinine hydrochloride, Weevers (1914) examined the depoisoning action of aluminium chloride towards a number of organic substances. The tissue used was red beet parenchyma, the exosmosis of the red pigment from the cells being taken as a criterion of toxic action. By this method it was shown that aluminium chloride could antagonise a number of organic poisons, namely, quinine hydrochloride, chloral hydrate, formaldehyde, amyl alcohol, ethyl alcohol, ether and chloroform. Antagonism was also observed between all these substances, except formaldehyde and ethyl

alcohol, on the one hand, and zinc sulphate, cobalt chloride and manganese acetate on the other. Antagonism was also observed between quinine hydrochloride and copper acetate, and between chloral hydrate and copper sulphate. Little or no antagonism was found between these compounds and sodium and potassium salts, nor between formaldehyde and any salt except aluminium, chloride. With each organic substance the depoisoning action of the trivalent aluminium ion was found to be greater than that of the divalent ions examined.

The antagonism between an aniline dye and an electrolyte was also demonstrated by Szücs (1910). The dye selected for most of Szücs' experiments was methyl violet, the intake of which by *Spirogyra* cells was examined by determining the time required for the cells to acquire a standard depth of colour. When, for example, potassium nitrate was present in a variety of concentrations (0·001 N to 0·08 N) in 0·0001 per cent. methyl violet solution, the time taken for the standard colouration of the cells to be acquired was found to vary with the concentration of the salt. The same was the case when calcium nitrate or aluminium nitrate was employed. In Table XLVI are exhibited the results obtained.

TABLE XLVI

Time taken for cells of *Spirogyra* to reach a definite intensity of colour when immersed in methyl violet solution containing various quantities of electrolyte. (Data from Szücs)

Concentration of methyl violet in per cent.	Electrolyte	Concentration of electrolyte in normalities	Time in minutes required for the acquisition of standard tint
0·0001	Potassium nitrate	—	2·0
0·0001	,,	0·001	3·0
0·0001	,,	0·01	5·0
0·0001	,,	0·08	8·25
0·0003	Calcium nitrate	—	0·83
0·0003	,,	0·001	2·25
0·0003	,,	0·01	8·50
0·0003	,,	0·04	8·50
0·0003	,,	0·09	9·17
0·0003	Aluminium nitrate	—	0·83
0·0003	,,	0·0005	8·0
0·0003	,,	0·0025	12·5
0·0003	,,	0·01	?19·0

Thus the extent of the antagonistic action is dependent both on the concentration of the electrolyte and on the valency of the latter,

the higher the valency the greater the antagonistic effect, while Szücs also found from experiments in which the concentration of the electrolyte (calcium nitrate) was kept constant and the concentration of dye varied, that the antagonistic effect also depended on the concentration of the dye. The time required for the cells to acquire a standard intensity of colour is not inversely proportional to the concentration; it was found instead that the product of time and concentration of dye in the external solution increased with increasing concentration of dye. But no doubt the relation between time required for the absorption of a' definite quantity of dye and the concentration of the dye in the solution, will depend on the quantity of dye selected. The inadequacy of this method of investigation has already been emphasized in an earlier chapter (Chapter x) and need not be further stressed here.

That the retardation of absorption of dye brought about by the presence of electrolyte is not due to an action between the dye and electrolyte outside the cells, becomes evident from a number of considerations. Practically the same concentrations of potassium, calcium and aluminium nitrates are required to precipitate methyl violet, so it would appear that the action of these three salts in bringing about molecular association of the dye must be approximately the same in each case, and consequently the coefficient of diffusion of the dye would be approximately the same in every case. Again, it was shown that yeast cells become stained with a 0·005 per cent. solution of neutral red in less than 10 minutes, while in the same solution containing aluminium chloride not until after the lapse of 48 hours. If this were due to molecular association and consequent reduction in the coefficient of diffusion, it would mean, assuming Herzog's formula (see Chapter IV), that the molecular weight of neutral red in presence of aluminium chloride is more than $2·3 \times 10^7$, which is obviously an impossibly big magnitude.

A consideration of the results collected in Table XLVI makes it clear that diluter solutions of electrolytes are relatively more active in antagonising the entrance of the dye than more concentrated solutions. According to Szücs his results with potassium nitrate agree well, and those with calcium nitrate and aluminium nitrate approximately, with the equation

$$t = \alpha C^{\frac{1}{n}}$$

where C is the concentration of salt and t the time taken for the dye to enter. Because of the similarity of this expression to the adsorption

equation, Szücs concludes that the extent to which the intake of dye is prevented depends on the adsorption of electrolytes by the protoplasm.

Further investigations on this question have been made by Endler (1912 *a*) who used only truly soluble dyes, particularly methylene blue, but also neutral red. Among the species employed were *Ulva lactuca, Nitophyllum punctatum, Spirogyra* spp. and *Elodea densa*. Endler's results deviated somewhat from those of Szücs. With a large number of chlorides and sulphates added singly to a solution of dye, he found that the rate of intake of dye was increased with increase in the concentration of the electrolyte present, but with further increase in concentration of the electrolyte the intake of dye decreased. Thus, with *Elodea densa* in a solution of 0·05 per cent. neutral red it was found that the maximum intake of dye took place when the chloride or sulphate of a number of metals was present in a concentration between 0·01 M and 0·05 M. Similar results were obtained with methylene blue, with which the order of inhibiting action of different salts was as follows: salicylate (complete prevention of dye intake with 0·001 M), aluminate (complete prevention of dye intake with 0·01 M) [citrate, tartrate], [chloride, sulphate], nitrate (recognisable uptake of dye with 0·4 M). Little difference was observed between the different salts of the same anions, with the exception that aluminium salts hinder the intake of dye more significantly than other salts examined.

Where the results of Szücs and Endler diverge, the explanation may be found in the fact that Szücs observed the time taken for a certain quantity of dye to be absorbed, while Endler was concerned with the total quantity of dye absorbed at equilibrium. Also the materials, both dyes and plant species chiefly used, were different in the two sets of experiments.

The action of a number of substances in influencing the intake of sulphonic acid dyes by perianth leaves of *Hyacinthus* has been examined by Collander (1921). He found the intake of cyanol and orange *G* was strongly hindered by a 2 per cent. solution of ether, although a 1 per cent. solution had only a little influence, while solutions containing 3 per cent. ether or more killed the cells in a short time. Chloral hydrate in concentrations from 0·5 to 1 per cent. also inhibited the intake of the dye, while 2 per cent. killed the cells in a short time.

Collander, using dyes in presence of phosphate mixtures of definite hydrogen-ion concentration, concluded that the hydrogen-

ion concentration greatly influences the intake of cyanol and orange
G by perianth leaves of *Hyacinthus*, the intake being furthered by
acidity and reduced by alkalinity. A 0·25 to 0·2 per cent. solution
of ammonium carbonate completely prevented the intake of cyanol
and orange G. These results fall into line with those of Harvey (1911)
and Endler (1912 b) who found the reverse influence of acidity and
alkalinity on the penetration of basic dyes. It is not clear how far
these results are connected with the general phenomena of antago-
nism.

Szücs (1910) found that the rate of intake of a basic dye, methyl
violet, or neutral red, is much reduced in presence of the acid dye
congo red. This is attributed to the formation of a compound of the
two dyes in the external solution: a compound which cannot enter
the cell. The non-entrance of the dyes in such cases is obviously not
comparable with the other cases already discussed.

Methods of making quantitative determinations of antagonism
by comparing amount of growth in plants in different solutions under
otherwise the same conditions, have been formulated by Osterhout
(1914 b, c, d). While such methods are undoubtedly of importance
from the point of view of the study of growth, they do not provide
a means of determining intake or permeability under different con-
ditions of composition of the external solution. One point of importance
emphasized by Osterhout (1914 d) to which reference may be made
is that the relative concentration of two salts in which antagonism is
greatest is not affected by dilution or concentration of the mixed
solution. This is certainly an important fact, but to state that this
law of direct proportionality "is in reality Weber's law" (Osterhout,
1916 c) appears to me an unwarranted extension of the scope of
Weber's law[1].

Exact quantitative data relating to the influence of one substance
on the intake of another are for the most part wanting. The best
available appear to be those of Szücs on the intake of methyl violet
in presence of various inorganic salts; these experiments have already
been described. It will be necessary to obtain a vast quantity of
further data before Szücs' view that antagonism in this case results
from adsorption of the inorganic salts by the protoplasm can be
accepted, especially as Endler's experiments on the intake of methy-

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[1] "Weber's law states that 'the just noticeable increase of a stimulus bears
a constant ratio to the original stimulus,' or 'two stimuli, in order to be dis-
criminated, must be in a constant ratio, the latter being independent of the
absolute magnitudes of the stimuli.'" (Flack and Hill, 1919.)

lene blue and neutral red suggest that the phenomenon cannot be so simply explained.

Osterhout (1916 *b, d*, 1917 *c*) has formulated what he calls a "dynamical theory of antagonism" based ultimately on the assumption that "an accurate measure of antagonism is furnished by the electrical resistance of living tissues." It is found that substances are of two kinds, those like sodium chloride, which bring about a fall in the electrical resistance of the thallus of *Laminaria Agardhii* and other plants, and those which, like calcium chloride, bring about an increase in electrical resistance followed very shortly (often within 15 minutes) by a decrease in resistance, which proceeds, as in the case of the first group of substances, to a maximum when the tissue is dead.

Osterhout supposes that two processes are involved, one producing a fall in resistance, the other a rise. He assumes that these two processes can be represented by the simple scheme
$$A \to M \to B,$$
where the substance A breaks down to form an intermediate substance M which itself breaks down to form B. It is further assumed that the resistance of the protoplasm is due to the intermediate substance M ("a substance at the surface of the cell which offers resistance to the passage of ions" (Osterhout, 1917 *c*)), and that the resistance of the tissue is proportional to the quantity of M + a constant equal to the resistance of the tissue when dead.

It is assumed that in *Laminaria* under normal conditions in sea water, the quantity of each of these substances remains constant. On transferring the mixture to a solution of sodium chloride or calcium chloride, or a mixture of the two, it is further assumed that the velocity constants of the two reactions $A \to M$ and $M \to B$ are altered. Then, if the reaction $A \to M$ is more rapid than the reaction $M \to B$, M will accumulate and the resistance will be raised until the supply of A becomes exhausted when M will form more and more slowly, so that ultimately it will decompose faster than it is formed, when the resistance will fall.

Values can be selected for the velocity constants of the two reactions so that on the assumptions already made curves between time and quantity of the substance M present can be constructed which agree with the curves of resistance of *Laminaria* in sodium chloride, calcium chloride or any mixture of the two salts. It is then found that as the quantity of calcium chloride in the solutions increases, the velocity constants of both the actions

$A \to M$ and $M \to B$ first rise and then fall, the minimum value naturally occurring in the mixture of salts in which the fall of resistance is slowest.

To account for these changes in the values of the velocity constants, Osterhout puts forward two alternative suggestions. According to the simpler one, the rate of the first reaction is increased by calcium chloride, which has a minimum influence when the molecular proportion of this salt to sodium chloride is $4\cdot76 : 95\cdot24$, that is, when the proportions are such that the resistance falls most slowly, while the rate of the second reaction is increased by sodium chloride, which also has a minimum effect when the ratio of calcium chloride molecules to sodium chloride molecules is $4\cdot76 : 95\cdot24$. "It makes little difference whether the value of the constant is increased by the salt, the effect passing through a minimum, or diminished by the salt, the effect passing through a maximum."

The alternative explanation supposes that the first reaction, $A \to M$, that is, the formation of the substance to which the tissue owes its resistance, is catalysed by calcium chloride, while both the first and second actions are retarded by the presence of a substance such as $Na_{20}XCaCl_{22}$ formed between sodium chloride, calcium chloride and a constituent X of the cell, and the action is supposed to be reversible. The quantity of this substance present is then governed by the law of mass action and can be calculated from the equation

$$\frac{C_{Na_{20}XCaCl_{22}}}{(C_{NaCl})^{20} \, C_X C_{CaCl_2}} = K,$$

where the symbols C_{NaCl}, C_{CaCl_2}, etc., represent the concentrations of the various substances concerned, and K is a constant.

When the maximum quantity of this negative catalyst is produced there will be maximum antagonism. Now, according to Osterhout, the position of maximum antagonism is independent of the absolute concentrations of the antagonistic substances, while the relative proportions of the salts giving a maximum quantity of the compound $Na_{20}XCaCl_{22}$ will depend on the concentration. To overcome this difficulty, Osterhout assumes that the negative catalyst is formed at the surface, the sodium chloride being supposed to migrate into the surface until the surface layer is saturated, so that with increased concentration of the salts either inside or outside the cell, the concentration in the surface layer remains constant.

It must be admitted that this is a rather tremendous superstructure of theory built on the foundation of the changes in electrical

resistance of *Laminaria* disks in solutions of sodium and calcium chlorides. While the explanations may be credible they are, for the most part, speculative, and on this account they do not call for consideration here. It may, however, be said that to regard changes in resistance as dependent on, and proportional to, the change in quantity of one substance in the cell, appears to the writer, for reasons cited in the previous chapter, to neglect the complexity of the system involved.

CHAPTER XIII

REVERSIBLE AND IRREVERSIBLE CHANGES
IN CELL PERMEABILITY

I T is commonly supposed that the permeability of the plant cell
is capable of undergoing changes, and statements to that effect,
and involving that point of view, are common in the literature of
the subject. But before discussing these changes it is necessary to
enquire what exactly is meant by the term "change in permeability."
In making this enquiry we are brought up at once against the difficulty
referred to in the first chapter, namely, our present very inadequate
analysis of the system involved. In the preceding chapters it has
already been necessary to call attention to the fact that the rate of
intake of a substance is not necessarily a measure of the permeability
of any or all of the cell membranes, as the rate of intake will depend
on the difference of concentration within and without the cell, and
so also on the position of the equilibrium attained in the intake. Nor
on the other hand can exosmosis be used as a measure of permeability.
To make the position clear let it be supposed that when a plant cell
is placed in a certain solution no diffusion out from the cell takes
place at all. Suppose the cell is now transferred to a second solution
into which a considerable exosmosis of substances takes place forth-
with from the cell. It would be generally said that the solute in the
second solution had brought about an increase in the permeability
of the cell, or of the protoplasm, or of the plasma-membrane. This
view is really based on the assumption that the protoplast or vacuole
is surrounded by a membrane which is changed physically or chemi-
cally by the solute in the external solution, so that the substances
within are now able to diffuse through the membrane. This might
indeed be the true explanation, but clearly others are possible. Thus
the solute in question might affect non-diffusible substances of com-
plex molecular constitution so that they broke down into a number

of substances of low molecular weight which can diffuse freely out of the cell. It is obvious that in such a case the change observed is not necessarily connected in any way with changes in permeability.

Nevertheless, it seems clear that substances may pass with different degrees of difficulty into or out from the cell under different conditions, even when the differences of concentration within and without the cell are the same, and it may be convenient to speak of any such difference which can be brought about by change in conditions as due to a change in permeability, although it must be emphasized that the term permeability is then not used in a strict sense (cf. Chapter I). Even then it is very questionable whether it is legitimate to speak of the permeability of the cell or of any part of it without reference to the substances concerned, for it is by no means certain that a change in permeability to one substance involves the same relative change in permeability to another substance (cf. Chapter V). In the following discussion the term "change of permeability" is used to indicate a change in the ease with which a substance will pass into or out from the cell unconnected with a change in the difference in osmotic pressure or of concentration within and without the cell. It should, however, be clearly recognised that in so doing the term permeability is used loosely and with no intention of accepting the point of view that the changes observed are entirely due to alterations in the permeability of a cell membrane.

Such changes may be reversible or irreversible. The latter appear always to be in the direction of increased permeability, with exosmosis of dissolved substances and, in the case of the turgid cell, of water, ending in complete loss of turgidity and in death. Such irreversible changes are produced by high temperatures, freezing and subsequent thawing, and toxic substances. It is possible they may be also brought about by rapid withdrawal of water resulting from immersion of a cell in a strongly hypertonic solution. The course of water loss during such an irreversible change in permeability is illustrated by the curves in Fig. 11 in Chapter X, showing the change in water-content of potato tuber immersed in solutions of ethyl alcohol of various concentrations. The exosmosis of electrolytes from the same tissue immersed in solutions of the same alcohol of various concentrations at a temperature of 20° C. is indicated by the curves in Fig. 16 which exhibit the increase in electrical conductivity (corrected for the presence of non-electrolytes) of 50 c.c. of external solution containing 20 disks of potato tissue, 17 mm. in diameter and 1·75 mm. thick. A comparison of the two sets of curves shows that the same concen-

trations of alcohol which bring about rapid exosmosis of water also bring about rapid exosmosis of electrolytes. It could be argued that the loss of water results from the loss of electrolytes and the consequent lowering of the osmotic pressure of the cell; in the present state of our knowledge it is safest to regard both as part of the phenomena of disintegration of the organisation of the cell characterising death.
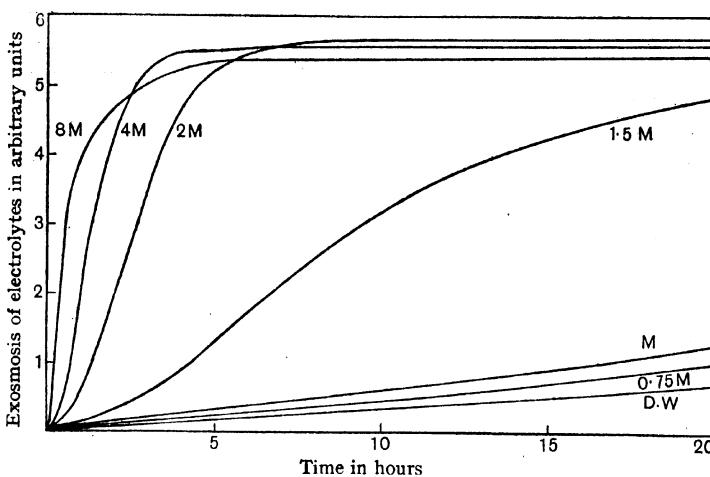
Fig. 16. Exosmosis of electrolytes from potato immersed in solutions of ethyl alcohol of various concentrations ($8M$ to $0.75M$) and in distilled water (D. W.). (After Stiles and Jørgensen.)

It was shown by Stiles and Jørgensen (1917 a) that the relation between the quantity of exosmosis and the time of action of a toxic substance can be expressed approximately by the equation

$$- B \log (1 - ks) = D \left(\frac{1}{A} e^{-At} + t - \frac{1}{A} \right),$$

where s is the concentration of the electrolytes that have exuded into the external solution after any time t and A, B, D and k are constants, this equation being that obtained when it is assumed that the rate of exosmosis at any time is proportional to the quantity of a substance in the cell which is decomposed by the toxic substance. The value of the constant A depends partly on the concentration of the toxic substance in the external solution; from the exosmosis-time curves obtained with different substances it is clear that whatever the relation between A and concentration may be it is not a linear one.

It has previously been mentioned that the electrical conductivity of tissue increases when acted upon by a toxic substance, the rate of increase giving a rough measure of the toxicity (Osterhout, 1912 *a*; Raber, 1920 *a*). Osterhout's elaborate theory of antagonism described in the last chapter is easily extended so as to explain the changes in electrical conductivity of tissue acted upon by a toxic substance (Osterhout, 1917 *e*).

The relation between concentration and toxicity was investigated by Wolfgang Ostwald (1907) in the case of a freshwater animal, *Gammarus*, this worker coming to the conclusion that the life-time *t* of the animal in a solution of concentration *C* is given by the equation

$$\frac{1}{t} = kC^p,$$

where *k* and *p* are constants.

The similarity of this equation to the adsorption equation led Ostwald to regard $\frac{1}{t}$ as proportional to the quantity of toxic substance adsorbed. The law has been shown to hold by Weevers (1912) for the toxic action of chloroform vapour on cells of the root of the red beet, the exosmosis of the red pigment being taken as an indication of the death of the cells. The same worker found (1914) that this relation only held within narrow limits in the case of the poisoning of the same cells with solutions of quinine hydrochloride and chloral hydrate. With more dilute solutions the cells live longer than the time indicated by Wolfgang Ostwald's equation. A similar divergence was observed by Szücs (1912) in his experiments on the time required for seedlings of *Cucurbita Pepo* to absorb enough copper sulphate to kill them, and by Nothmann-Zuckerkandl (1912) in experiments on the action of alcohol on protoplasmic streaming in *Vallisneria*.

Several arguments against Ostwald's conclusion have been put forward by Szücs, who showed that the former writer's view that the time taken to kill is inversely proportional to the quantity of poison adsorbed involves several assumptions. It assumes in the first place that equilibrium between the quantity adsorbed and the outer medium appears instantaneously, for otherwise the life-time will be increased. In the second place it is assumed that small quantities have the same effect ultimately as larger ones, but require more time for the effect to be observed. Thirdly, it is assumed, since the adsorption equation only refers to an equilibrium condition, that the toxic effect is only produced when the equilibrium is reached.

These various assumptions are unjustified by the facts. With regard to the first assumption, equilibrium in the intake of copper sulphate was shown by Szücs to come about very slowly. With regard to the second assumption, it is not true that small quantities have the same effect as larger ones if they are given the necessary time to act, for the intake of small quantities of a poison may not lead to death. That this is not due to the washing out of the poison into the surroundings is shown by the fact that it takes place with heavy metals which are supposed to form irreversible compounds with the cell proteins, and that if plants are grown with their roots in a poisonous solution and removed from it before the lapse of a definite critical time they survive. Finally, with regard to the third assumption, Szücs found that with very poisonous substances death may occur before the equilibrium condition is reached.

For these reasons Szücs held that Ostwald's theory could not be maintained in its original form. Szücs himself appeared to incline to the opinion of Morawicz (1910) that the same quantity of material must be absorbed to bring about death. Szücs himself found the time required for seedlings of *Cucurbita Pepo* to absorb enough copper sulphate to kill them, by maintaining their roots in the copper sulphate solutions for definite times and then testing their vitality by examining their power to react to the stimuli of light and gravity. He then found that where t is the time required for the seedlings to take up enough copper sulphate to inhibit the geotropic reaction,

$$\frac{1}{t} = kC^p,$$

k, C and p having the signification previously assigned to them.

But these considerations on the relation between the concentration of toxic substances and time taken to kill, do not help us very much in determining the influence of concentration in bringing about a change in permeability. They have, however, this bearing on our subject, that, according to the view involved in Wolfgang Ostwald's theory, a substance that produces irreversible changes in permeability leading to death, would do so at once, whereas according to the view of Szücs the action of the toxic substance might only become irreversible after a definite critical period of immersion, varying in length with the concentration according to the equation cited above.

It is at least possible that all changes resulting in increased permeability of the cell are reversible if the condition producing the change is not allowed to act for more than a certain time.

It is generally supposed that all living cells in their normal condition undergo changes in regard to the ease with which substances pass into and out from them. These are sometimes spoken of as functional (Bayliss, 1915) changes in permeability. As these changes are reversible they can obviously take place in either direction, that is, so as to increase or diminish the capacity of substances to pass into or out from the cell. In the former case, if the action is prolonged, we may expect the change to become ultimately irreversible.

Factors which may bring about changes in permeability are those of the environment of the cell, namely, temperature, light and the composition of the external medium. The changes produced by various factors will now be considered.

EFFECT OF TEMPERATURE ON PERMEABILITY

The influence of temperature on the rate of intake and exosmosis of water by plant cells, and on the permeability of cells to water, has already been considered in Chapter x, and the influence of temperature on the rate of intake of dissolved substances has been discussed in Chapter xii. The effect of temperature over the range 0° C. to 30° C. is to increase the rate of intake of both water and dissolved substances, although the magnitude of the temperature effect varies considerably. A good case can be made out for regarding the change in the rate of intake of water into the vacuolated cell with change in temperature as due to a change in permeability of the protoplasm. That the same is the case with dissolved substances is not in any way evident. Within the range of temperatures indicated, changes related to changes of temperature are certainly reversible, even if the conditions are continued over comparatively long periods of time, but if the temperature rises too high, or if the tissues are allowed to freeze, the tissues or cells quickly disorganise owing to other changes which as far as we know may have nothing to do with permeability.

EFFECT OF LIGHT ON PERMEABILITY

The influence of light on the intake and excretion of salts has already been dealt with in the preceding chapter. Such changes in relation to light appear undoubtedly to be reversible. Here again they can only be spoken of as changes in permeability if the latter term is used in a loose sense.

Haupt (1902) concluded that light influences the permeability of the cells of extra-floral nectaries in *Euphorbia* and *Vicia*. He found excretion of sugar only took place from the cells when illuminated,

while in the dark sugar was absorbed. Since the behaviour is observed in plants that have been maintained for days in absence of carbon dioxide it is concluded that the behaviour is not related to carbon assimilation, but is due to changes in the permeability of the cells to sugar. But this explanation appears to me hardly to be in agreement with the facts, for a mere change in permeability of a membrane will not account for the reversal in direction of the movement of the solute. A change in the physical or chemical constitution of the cell contents, brought about by a photochemical reaction, appears a much more credible explanation of the observed facts.

INFLUENCE OF DISSOLVED SUBSTANCES ON PERMEABILITY

In addition to the antagonistic effects described in the last chapter there is a certain amount of evidence that substances in solution external to plant cells influence the rate at which substances pass into or out from plant cells. The action of toxic substances in this respect has already been discussed. Apart from these undoubted cases there are a number of observations on record which are alleged to indicate the influence of dissolved substances in altering permeability. Thus, Wächter (1905) found that the passage of sugars out from the bulb scales of the onion was prevented by the presence of potassium nitrate in the liquid external to the plant. A rather similar observation is that recorded by Bayliss (1915) to the effect that while a $0.31 M$ solution of sodium chloride brings about exosmosis of the pigment from the cells of the root of the red beet, no such exosmosis takes place into a solution containing the same concentration of sodium chloride and also 0.17 per cent. of calcium chloride. Both these observations can be explained on the basis of a reduction of permeability of the cell to a cell constituent, the reduction being brought about by the presence of a solute in the external solution. In the second case the observation can be brought into line with the antagonistic effects noted in the last chapter.

Fluri (1909) thought he had obtained evidence that aluminium salts brought about an increase of permeability of plant cells to glucose and a number of salts. When filaments of *Spirogyra* were immersed for two or three days in a 0.01 per cent. solution of aluminium sulphate or nitrate, the cells were no longer plasmolysable in hypertonic solutions of glucose or a number of salts. Fluri explained this as due to the increased permeability of the cell to these substances. On transferring the filaments back to water the cells recover their original condition, so that the effect is reversible.

However, investigations of Stoklasa (1911) and others, and particularly the critical research of Szücs (1913) on this subject, indicate that this explanation is not correct, for if it were, the following consequences should result.

1. Cells plasmolysed in a hypertonic solution should become deplasmolysed after addition of an aluminium salt to the external solution.

2. The increased permeability of the protoplasm should condition increased exosmosis and consequent loss of turgor.

3. As they can enter the cell more rapidly, toxic substances ought to exhibit their effects more rapidly in presence of aluminium salts.

4. Substances which are microscopically recognisable ought to be clearly discernible by microchemical tests if they enter the cell in such amount that plasmolysis is prevented.

5. The rapid entrance of strong solutions ought to have toxic effects which would condition exosmosis.

Szücs could observe none of these consequences of Fluri's theory of the action of aluminium ions. On the contrary, he found by centrifuging filaments of *Spirogyra* treated with an aluminium salt, that the action of aluminium ions is to fix the protoplasts, which remain in position under this treatment, while normal living protoplasts collect in the ends of their cells. Aluminium chloride, sulphate, nitrate and acetate all appear to behave in the same way, while yttrium and lanthanum salts also appear to have the same action. On returning the cells after treatment to water, they recover their original state. There is no doubt that the effect is reversible. If the aluminium ion is allowed to act for a longer time, and if it is in sufficient concentration, the protoplast becomes "loosened" again, and then collects in the ends of the cell on centrifuging, in the same way as in a normal living cell. The fixing action can be observed in *Spirogyra* immersed for only a minute in a solution of aluminium nitrate as dilute as 0·0064 N. The greater the concentration the more rapidly both the fixing action and the subsequent "loosening" take place. It should be noted that aluminium ions do not exert this fixing action on anthocyanin-containing cells of a number of different species examined by Szücs.

Harvey (1911, 1913), from experiments in which cells were first impregnated with neutral red (cf. Chapter XI), concluded that sodium hydroxide does not readily enter cells of *Elodea*, *Spirogyra*, *Paramœcium* and eggs of various echinoderms, but that sodium salts in the external solution render the cell permeable to sodium hydroxide.

It is not clear, however, how far this result is to be attributed to toxic action.

S. C. Brooks (1916 *b*) treated strips of peduncle of *Taraxacum* with solutions of respectively 0·22 *M* sodium chloride, 0·17 *M* calcium chloride and 0·05 *M* cerium chloride for 20 or 25 minutes, then transferred the tissue to distilled water and measured the increase in electrical conductivity of the latter. He assumed that exosmosis during the first 30 minutes is due to diffusion into the water of the salt previously absorbed, and that subsequent exosmosis can be regarded as due to exosmosis of electrolytes from the protoplasm. Since it was found that after 30 minutes exosmosis from tissue that had been treated with calcium chloride was less than that from tissue which had not been in contact with any salt solution, it was concluded that calcium chloride brings about a decrease in permeability of the tissue, while as from tissue treated with sodium chloride exosmosis after 30 minutes was greater than from the control which had not been in contact with salt solution, it was concluded that sodium chloride brings about an increase in permeability. Cerium chloride was regarded as bringing about first a decrease in permeability which is followed by an increase.

To the present writer these conclusions appear of little value for the following reasons. The recorded observations are few in number, the recorded differences are small, and it is not shown that they lie outside the limits of experimental error. It is necessary to show this, especially as electrical conductivity can be used only as an approximate, but not as an accurate, measure of the exosmosis of electrolytes, while it is unlikely that diffusion of the experimental salt out of the tissue should end, and that of other substances in the cells begin, at any particular moment, or at the same time with different salts.

Experiments with the tissue tension method and the diffusion method described in the last chapter led Brooks to similar conclusions, namely, that salts of univalent kations (and also sucrose) increase permeability, while salts of divalent and trivalent kations at first bring about a decrease in permeability. But these methods also are not free from serious objections, and the legitimacy of the conclusions drawn appears to the present writer very doubtful (cf. Chapter XI).

As indicated in the last chapter Osterhout (1912 *a*, *b*) found that thallus of *Laminaria* when transferred from sea water to sodium chloride having the same conductivity as sea water, underwent a gradual loss of electrical resistance until the tissue was quite dead.

A number of salts were found to act in the same way, namely, the chlorides of potassium, cæsium, rubidium, lithium, ammonium and magnesium[1]; sodium bromide, iodide, nitrate, sulphate and acetate. On the other hand the salts of bivalent metals examined (magnesium, calcium, barium, strontium, manganese, cobalt, iron, nickel, zinc, cadmium and tin) produce first a rise in resistance, then a fall (Osterhout, 1915 *d*); the same is the case with salts of trivalent and tetravalent metals (Osterhout, 1915 *e*). The same effect is produced by ether, chloroform, chloral hydrate and alcohol (Osterhout, 1913 *a*, 1916 *f*), by potassium cyanide (Osterhout, 1917 *a*) and by sodium taurocholate (Osterhout, 1919 *b*).

On the assumption that electrical conductivity is a measure of permeability these results are interpreted as indicating that the substances of the first group bring about an increase in the permeability of the cell, while those of the latter bring about first a decrease in permeability followed by an increase. For the same reasons small quantities of sodium hydroxide are held to increase permeability (Osterhout, 1914 *g*) while hydrochloric acid is considered to produce rapid decrease of permeability followed at once by rapid increase (Osterhout, 1914 *h*).

If the tissue is only allowed to remain in the single salt solutions for a short time and is then returned to sea water, the original resistance of the tissue is regained. This is regarded by Osterhout (1912 *b*, 1915 *b*) as indicating reversible changes in permeability.

Reasons why electrical conductivity cannot be regarded as an accurate measure of permeability have already been advanced in Chapter XI and need not be repeated here. Also it seems to the present writer meaningless to speak of the permeability of the cell as a quantity with a definite value which can be accurately measured, unless the substance, the penetration of which is in question, is also stated,. for there is no evidence that if the permeability of the cell to one substance changes, its permeability to all other substances changes in the same proportion. Osterhout's theory of antagonism described in the last chapter has been extended to include reversible changes of electrical conductivity (Osterhout, 1920 *a*, *b*), but as it is highly speculative, it will not be dealt with further in this place.

Tröndle (1920) investigated the effect of acids on the permeability to sodium chloride of the cells of the leaf of *Buxus sempervirens* by a plasmolytic method in which were recorded the concentrations of sodium chloride in which deplasmolysis just took place after various

[1] But magnesium salts appear in Osterhout's second group.

times of immersion. It was found that after immersion for five minutes in 0·01 N oxalic acid or 0·005 N hydrochloric acid the plasmolysing concentration of sodium chloride rose more rapidly than after a preliminary immersion for five minutes in tap water. The rise in the value of the limiting plasmolysing concentration is attributed to the absorption of sodium chloride and hence it is concluded that dilute acid has the effect of increasing the permeability of the cells in question to sodium chloride.

The observation of Puriewitsch (1898) that exosmosis of sugars or other soluble food reserve substances from storage tissue is inhibited in absence of oxygen or in presence of anæsthetics, is regarded by Livingston (1903) as probably a case of the influence of a dissolved substance on the permeability of a cell membrane. Yet to me Puriewitsch's original explanation, namely, that the absence of oxygen or the presence of anæsthetics influences the enzymes present so that the stored food material is not converted by enzyme action into a diffusible form, appears at least as probable. Livingston finds it difficult to understand how enzyme action could enter into the question in cases where the food reserve is in a soluble form as in the case of sugar in onion and beet root. It might, however, be the case that there is here a transformation of disaccharides or more complex sugars into the more easily diffusible hexoses or pentoses.

The influence of various narcotics on the absorption of salts by leaf cells was examined by Tröndle (1920) by the plasmolytic method used by him to test the action of acids on permeability. Assuming the legitimacy of the method, which is in doubt, it was found that previous immersion for 20 minutes in 0·5 per cent. chloral hydrate reduced the rate of absorption of sodium chloride by leaf cells of *Buxus sempervirens*, while immersion of the same cells for the same time in 1 per cent. chloral hydrate or 3 per cent. (by volume) ether at about 15° C. completely inhibited the intake of salt. The absorption of potassium chloride by leaf cells of *Acer platanoides* was inhibited by previous immersion for 15 minutes at about 21° C. in 1 per cent. chloral hydrate, and similarly the absorption of sodium nitrate by leaf cells of *Lupinus albus* was inhibited by immersion for 20 minutes in the same solution. From these results Tröndle concludes that narcotics reduce the permeability of cells to salts, even to complete impermeability[1].

[1] By using the plasmometric method S. Prát ("Plasmolysis and Permeability II," *Preslia*, **2**, 90–97, 1922) has found an indication of a reduction of permeability of *Spirogyra* cells to potassium and sodium chlorides as a result of preliminary treatment of the cells with a dilute solution of aniline.

EFFECT OF CHANGES IN TURGOR

To account for the exudation of water or solution from living cells in the case of water pores and nectaries, and in the phenomena of bleeding and root pressure, it has been suggested that a chahge in the permeability may be involved which is brought about when the turgor pressure has reached a certain amount. It is certainly conceivable that a stretching of the protoplasmic envelope might render it more permeable. Livingston (1903) thus thinks that as water passes into the cells in question the turgor pressure may rise to a critical point when a change in permeability takes place as a result of which cell sap exudes through the protoplasm. As a result of this the turgor pressure is reduced and the cellulose wall contracts. If the change in permeability is uniform throughout the whole of the protoplasm, exudation will only take place to any extent into the cavity of the pore of the water stoma or the cup of the nectary, as the turgor pressure of the surrounding cells will oppose the passage of water into them.

After the cell wall and protoplasm have contracted so that turgor pressure is below the critical value, the protoplasm regains its semi-permeable property, water passes in from the neighbouring cells, and the turgidity increases until the critical value of the turgor pressure is again reached. During this period reabsorption of the water previously extruded is prevented because evaporation has concentrated the extruded solution so that it has now a higher osmotic concentration, and would therefore tend to extract water from the cell. It must be supposed on this (and perhaps not only on this) view of the action of water pores and nectaries, that the solutes lost from the vacuole in exudation are replaced by secretion in the protoplasm.

A similar mechanism can be supposed to act in the case of the exudation of water from cut stems and leaves, and in the phenomenon of root pressure.

It must be emphasized that this theory was only put forward by Livingston as a tentative one. It is possible that no change in permeability is involved in the phenomena of exudation, bleeding and root pressure, and references to theories in which this latter point of view is taken have already been noticed in Chapter IX.

EFFECT OF WOUNDING

Tröndle (1921) investigated the effect of wounding on the permeability of cells of the young roots of *Pisum, Vicia, Lupinus* and *Allium* by the method used by him to investigate the effect of acids and narcotics on permeability, and already mentioned in this chapter,

that is, by determining the limiting concentration of sodium nitrate and potassium chloride required to produce plasmolysis of these cells after the lapse of different periods of time. Assuming the legitimacy of the method, which is doubtful, it was found that injury decreased the rate of intake of the salts employed for plasmolysis, whence it is concluded that wounding decreases the permeability of the protoplasm of these cells to the salts in question.

SEASONAL CHANGES IN PERMEABILITY

Fitting (1915) found by the deplasmolytic method used by him, that salts were absorbed by the cells of the leaves of *Rhœo discolor* much more slowly in winter than in summer; he found the same thing with glycerol (1919).

It is evident from what has already been written that the cell may vary under different conditions with regard to its capacity for absorbing or excreting both water and dissolved substances. In the case of the vacuolated cell such changes in the rate of the passage of water from external liquid to vacuole or *vice versa* may, with constant difference in osmotic concentration of the internal and external liquids, be regarded as due to changes in the permeability of the cell membranes (cell wall + protoplast) to water, although even this rests on the assumption of the complete correctness of the simple osmotic view of the plant cell, which has already been shown to be inadequate (cf. Chapter IX).

The question of changes in permeability to dissolved substances is a much more difficult one. That changes in permeability are spoken of at all in this connexion is due to the acceptance of the membrane theory, and it must not be forgotten that other explanations are possible which do not involve passage of substances through a membrane (Moore and Roaf, 1908). With regard to the irreversible changes in permeability leading to the death of the cell, Szücs (1912) is of opinion that the so-called "increase in permeability" brought about by narcotics and other compounds, and indicated by exosmosis of tannin from plant cells and the pigment from red blood corpuscles, has nothing to do with permeability because the phenomena observed are not connected with vital processes but are due to post-mortal changes.

Yet even if the exosmosis resulting from the action of toxic substances is not in the first place attributable to post-mortal changes,

it does not follow that such exosmosis is due to increased permeability of the cell membranes to the solutes of the cell. It may, for instance, result from a breaking down of complex and indiffusible substances into simpler and diffusible ones. Similarly, the so-called reversible changes in permeability may be attributed to reversible changes in molecular associations rather than to changes in the permeability of the cell membranes. Which is the correct view cannot be decided in the present state of our analysis; it can only be determined by further work.

CHAPTER XIV

THEORIES OF CELL PERMEABILITY

I T will be clear from the review in the preceding chapters of the data at present available with regard to the permeability of plant cells, that our information is far from complete, and, as far as quantitative data are concerned, fragmentary. Nevertheless, in spite of the insufficiency of information, there is no shortage of theories of cell permeability. It is one of the weaknesses of the theories of cell permeability that they are for the most part not even based on the whole of the scanty information available, but only on one particular set of observations which fit the particular theory. Under these circumstances perhaps none of the theories are of any great value, and no attempt will be made here to summarise in any detail all the theories that have been put forward to account for certain facts of cell intake or excretion. Those theories will chiefly be considered which have come into prominence during recent years, and which, in the opinion of the writer, may be perhaps of some use, in that they may stimulate to further work.

Two questions which have been discussed in earlier chapters are obviously of first importance in regard to the mechanism of permeability in living cells. These are (1) the purely physico-chemical question of the permeability of membranes discussed in Chapter v, and (2) the plasma-membrane discussed in Chapter viii. From the discussion on the plasma-membrane it must be admitted that the evidence for the presence of a membrane which acts as a semi-permeable membrane separating the bulk of the protoplasm from the external solution (supposed to be present as such in the cell wall) is not by any means convincing, although to regard the whole body of the protoplasm in the vacuolated cell as a more or less semi-

permeable membrane separating the external solution from the vacuole has more in favour of it.

Most of the propounders of theories of cell permeability assume a semi-permeable plasma-membrane forming the external layer of the protoplasm in all cells, and if this were the case, it might perhaps be possible to find an explanation of cell permeability that would hold in all cases. But if this supposition is erroneous it is clear that we cannot expect a theory which explains the passage of substances from the outside of the cell through the protoplasm into the vacuole, necessarily to explain also the intake of the substance by the protoplasm, that is, the membrane.

To those who believe in a more or less semi-permeable plasma-membrane limiting the protoplasm externally, the three theories of membrane permeability described in Chapter v are obviously applicable to the penetration of substances into the cell, and we do actually find that all three theories, the sieve theory, the solution theory and the chemical combination theory, have been put forward in some form or another as theories of cell permeability. But to those who do not accept the presence of such a plasma-membrane, these theories in their simple form obviously cannot account for the intake of substances by meristematic cells or excretion from them, while in the case of vacuolated cells they can at most only account for the passage of substances into the vacuole and not for their uptake by the protoplasm. This essential dependence of theories of cell permeability on belief or disbelief in the existence of a plasma-membrane of restricted permeability has not always been sufficiently emphasized in discussions on the mechanism of absorption by cells, even if it has been fully realised.

Since some theories of cell permeability rely for some considerable measure of support on observations on the intake of dyes by living cells ("vital staining") it will be as well to preface a consideration of the individual theories with a brief summary of the main facts of dye intake.

The work of Pfeffer and subsequent investigators suggested that while plant cells rapidly absorb basic dyes, acid dyes are in general absorbed not at all or only to a slight extent. Overton (1899, 1900), whose theory of permeability will be discussed later, came to the conclusion that only dyes soluble in lipoid substances enter the cell. Basic dyes are, however, on the whole, soluble in lipoid substances, while acid dyes are not. It was later found by Höber and Kempner (1908) and Höber and Chassin (1908) that many acid dyes are taken

up by the epithelial cells of the kidney and accumulate there; nevertheless, some acid dyes are not taken up. Höber and his co-workers came to the conclusion from their experimental results that when a dye is not absorbed by the epithelial cells of the kidney it is highly colloidal; whereas if a dye is only slightly colloidal or semi-colloidal it is easily absorbed.

In the next year Ruhland (1908 *a, b*) came to the conclusion from experiments with *Spirogyra*, that whatever the degree of dispersity of the dye, the cells in question readily absorb a basic dye but not an acid dye. In the same year Höber (1909) examined the capacity of 34 dyes to stain the epithelial cells of the kidney and came to the same conclusion as he had done previously, namely, that those dyes which do not stain these kidney cells are unable to do so on account of their low degree of dispersity. He rightly pointed out that his results only hold for kidney cells and were not necessarily applicable to cells in general.

In further work which will be dealt with in some little detail later in this chapter, Ruhland (1912 *b*, 1913 *a, b, c*) examined the intake of a great number of dyes, both acid and basic, by a different method and as a result changed his point of view and now holds that it is solely the size of the particles of the dye which determines whether the dye is taken up or not.

The importance of the hydrogen-ion concentration of the cell contents is emphasized by Bethe (1916, 1922) and Rohde (1917), who find that the capacity of living cells to absorb dyes depends in high degree on the reaction of the external medium and of the cell contents. Acid reaction in the interior of the cell favours the accumulation of acid dyes and acts against staining by basic dyes, while inversely, basic dyes are extraordinarily strongly absorbed by cells with an alkaline reaction while acid dyes scarcely stain such cells at all. Bethe (1922) adduces in support of this view, experiments in which basic and acid dyes and one amphoteric dye were allowed to diffuse through parchment paper under different conditions of hydrogen-ion concentration. With acid dyes diffusion is furthered by an acid reaction and reduced by an alkaline one; with basic dyes the reverse is the case. Similarly, if the dye is allowed to diffuse from an aqueous solution through parchment paper into a protein, the dye accumulates in the solution when an acid dye diffuses into an acid protein sol or when a basic dye diffuses into an alkaline protein sol, but not when the reaction of the sol is acid and the dye a basic one, nor when the reaction of the sol is alkaline and an acid dye is employed.

With these prefatory remarks we are now in a better position to consider some of the theories of cell permeability.

THE ULTRAFILTRATION THEORY

The ultrafiltration theory of cell permeability appears to be a direct application to the cell of the sieve theory of membrane permeability. In its present form it is founded on the work of Küster (1911) and Ruhland (1912, 1913 *a, b, c,* 1914) on the staining of various plant cells by a considerable number of dyes. The method chiefly employed was introduced by Küster and consists in placing the cut surface of shoots in a solution of dye so that the solution is carried through the vascular bundles from which the dye, if it is capable of penetrating living cells, will pass into the latter. Using this method Ruhland examined the penetration of 89 acid dyes, chiefly into the tissues of young plants of *Vicia Faba,* and came to the conclusion that a complete parallelism exists between penetrability and diffusivity of the dye, the latter being examined by following the diffusion of the dye through a gelatin gel. The diffusivity is supposed to run parallel to the degree of dispersion (cf. Chapter IV), whence it is concluded that with acid dyes the capacity of penetrating into plant cells depends entirely on the degree of dispersion of the dye.

Ruhland also examined the intake of 30 basic dyes by epidermal cells of the bulb scales of *Allium Cepa* and by cells of *Spirogyra,* and found that of these a few, namely, Victoria blue *R* and *B,* Basler blue *R* and *BB,* gallamin blue and night blue, were not absorbed, while two others, diazine green and Victoria blue *R,* were only absorbed slowly. All these dyes were also found to diffuse slowly through a gelatin gel.

In some cases the intake of acid dyes by cells of the bulb scales of *Allium Cepa* and of the pith of the stalk of *Helianthus annuus* could be made plain after immersion of the tissue in the dye by plasmolysing with a strongly hypertonic solution of sugar or a salt so that a big contraction of the protoplast resulted, with a consequent intensification of the colour. Küster (1918) has also obtained similar results in regard to the intake of acid dyes (acid fuchsin, light green *FS* and orange *G*) by cells of the pith of *Coleus hybridus.*

The fact that basic dyes accumulate much faster than acid dyes is regarded by Ruhland as not related to permeability, but to be due to the combination of basic dyes with tannic acid (cf. Chapter XI) so that a considerable apparent difference in concentration of the dye on the two sides of the plasma-membrane is maintained.

Ruhland thus comes to the conclusion that the entrance or non-entrance of a substance into the cell is related entirely to the magnitude of its molecules or molecular aggregates, the plasma-membrane acting exactly as an ultra-filter.

Ruhland's theory has been subjected to criticism by Höber and Nast (1913). It will be recalled that Ruhland found a few highly colloidal basic dyes that would not penetrate the cells examined. Höber and Nast, on the other hand, found that all these dyes will stain living animal cells with the exception of gallamin blue, and this dye, according to Höber and Nast, is an acid and not a basic dye. These dyes are not very soluble and are easily precipitated by electrolytes, and Höber and Nast consider it possible that the dyes were precipitated before they reached the plasma-membrane, possibly by the electrolytes in the cell walls. In any case the ultra-filter theory is not supported by the work of Höber and his collaborators on animal cells.

The ultrafiltration theory has been criticised by Collander (1921) from another point of view. The last-named writer points out that the cells which take up the dye when Küster's method is used are those in the neighbourhood of the vascular bundles, and Collander's work on the intake of sulphonic acid dyes has shown that these cells may have a quite abnormally high capacity for absorbing acid dyes. If the theory were sound all cells should absorb acid dyes of a high degree of dispersion, but this has been shown by Collander not to be the case, while the earlier experiments of Ruhland (1908 *a, b*) himself indicate the same thing. It is clear that the ultrafiltration theory cannot be accepted as a complete and general theory of cell permeability.

THE LIPOID THEORY

The lipoid theory of cell permeability propounded by Overton (1895, 1896, 1899, 1900, 1901) is a solution theory of permeability, assuming as it does that the penetrating capacity of different substances runs parallel with their solubility in lipoid substances of which the plasma-membrane is supposed to be composed.

Overton founded his theory on experiments with a wide range of tissues and a large number of different substances including many dyes and other organic compounds of different groups.

As already mentioned, most acid dyes are insoluble in lipoid substances, but Ruhland states that some sulphonic acid dyes are soluble in lipoids and do not stain living cells, and three acid dyes, cloth red 3*GA*, true red *A* and wool violet *S*, were found by Höber

(1909) to be soluble in lipoid substances, but yet did not stain most cells. Höber showed that wool violet may be actually absorbed but undergoes a change in the cell resulting in decolorisation, as this can be effected by treatment of the dye with fresh frog's liver. Küster (1911) found all these dyes were absorbed by some, although not all plant cells. However, Collander has shown that cells of sugar beet and *Elodea* do not absorb wool violet *S* with any rapidity. There appears to be some doubt whether this dye should be regarded as a typical lipoid-soluble dye, as an experiment made by Collander of the partition of the dye between water and a saturated solution of cholesterol in benzene resulted in the dye going in greater quantity to the water.

·Höber also points out that in another group of acid dyes, the phthaleins, there are a number which are soluble in lipoid substances but which do not stain living cells, namely, rose bengal, cyanosin, erythrosin and gallein.

Ruhland also states that new blue *R* is a dye insoluble in lipoid substances, but which stains living cells. Even methylene blue, one of the most easily absorbed dyes, prefers water to lipoids (Loewe, 1912). However, Höber and Nast found new blue *R* was soluble in a solution of cholesterol in turpentine. The absorption of many sulphonic acid dyes by certain plant cells as shown by Küster's method, appears to tell against the lipoid theory, as these dyes are in general insoluble in lipoid substances.

It is clear that a simple lipoid theory will not explain the behaviour of all cells to all dyes, but the principal difficulty arises from the intake of inorganic salts which are as a rule insoluble in lipoid substances. Consequently the theory has to admit that substances insoluble in lipoids may, under certain circumstances, be able to penetrate into the cells. Hence we find solution theories put forward in which the plasma-membrane is regarded as a mosaic (Nathansohn, 1904 *a*) or a colloidal complex (Lepeschkin, 1910 *a, b*, 1911 *a, b, c*) (cf. Chapter VIII) in which lipoid substances are present, by solution in which dyes, narcotics and other lipoid-soluble substances enter the cell, and in which there is also an aqueous phase through which water-soluble substances such as inorganic salts, acids and bases, and sugars, are able to diffuse into the cell.

The lipoid theory is at best an imperfect theory, only professing to indicate, as Collander puts it, what substances will enter all cells easily under all conditions. But even thus restricted the theory appears to break down, for, as already indicated, there are substances

soluble in lipoid substances which have been found not to enter living cells.

Boas (1921, 1922) made observations on the action of saponins on yeast cells and concluded that his results support the lipoid theory. He found that saponins in low concentrations bring about an increase in the rate of fermentation by yeast, although in high concentrations fermentation is retarded. This action is explained on the ground that saponins attack lipoids in the plasma-membrane so that the permeability is greatly increased and because of this sugars can be more rapidly fermented. The argument is not very convincing.

A modification of the lipoid theory recently proposed by Nirenstein (1920) as a result of work on *Paramœcium caudatum*, need not detain us long. According to this theory the living cell behaves as if it were a lipoid solvent containing a certain amount of fatty acid and organic bases soluble in fats, which between them are responsible for the uptake of substances, acid dyes, for example, being absorbed by the bases, such as diamylamine, in which they are soluble, and basic dyes by the fatty acids, such as oleic acid, in which they are soluble. Collander points out that this theory is not directly applicable to plant cells because accumulation of dyes takes place in the cell sap, but it might be possible to regard the plasma-membrane as possessing the solvent properties of *Paramœcium* protoplasm. However, Collander examined seven of the acid dyes which Nirenstein had found soluble in diamylamine and which stain living *Paramœcium*, but none of these dyes were taken up to any extent by plant cells. Nirenstein's theory is thus quite unacceptable in regard to plant cells.

THE COLLOID PRECIPITATION THEORY

A theory that permeability of protoplasm to any particular salt is dependent on the capacity of the salt to precipitate the colloids of the protoplasm has been put forward recently by Kahho (1921 *d*). This worker finds (1921 *a, d*) that the influence of salts on the coagulation of cell colloids by heat runs parallel with the penetrability of the salts as determined by the plasmolytic or tissue extension methods. The series obtained when kations and anions are arranged in order of their penetrability have been stated in the last chapter. These series are the reverse of the lyotropic series indicating order of capacity for precipitating proteins. If kations and anions are arranged in order of toxicity or of capacity for coagulating plant protoplasm, they fall into the same reverse lyotropic series (Kahho, 1921 *b*) while kations of the heavy metals appear to conform to the

same rule (Kahho, 1921 c). Kahho explains all these relations on the view that toxic action depends on power of penetration, while power of penetration depends in inverse fashion on capacity for coagulating certain of the cell colloids, probably the lipoid constituents of the protoplasm. Thus the reason why a calcium salt reduces the toxicity of a sodium salt is that the calcium coagulates the lipoid constituents of the limiting layer of the protoplasm, which according to Hansteen-Cranner (1919, 1922) penetrates into the interstices of the cellulose-pectin ("cellulose-hemicellulose") colloidal network of the cell wall. This coagulation of the lipoid constituents renders the outer layers of the protoplasm less permeable to the salts in the external solution so that the entrance of these salts with their consequent toxic action is prevented and the coagulation of the proteins of the protoplasm is thereby prevented.

This theory is attractive and Kahho marshals his own experimental data well in its support and adduces a number of observations by other workers as evidence on behalf of his theory. It must nevertheless be admitted by an unprejudiced critic that the experimental basis of the theory is still rather frail, the essential facts, namely, those relating to the actual penetration of different salts, having been obtained by the tissue extension method of Lundegårdh, the validity of which, for reasons advanced in an earlier chapter, is open to a certain degree of doubt, and which should certainly be confirmed by means of other methods of determining salt absorption. Considerably more work on the location and properties of the cell colloids in the actual species used would also appear desirable.

THE VISCOSITY THEORY

It has been suggested by Spaeth (1916) that the permeability of the plasma-membrane is determined by its viscosity, which is itself determined by the degree of dispersion of the colloids contained in it. On this view increased permeability is to be regarded as due to increase in rate of diffusion consequent on decrease in viscosity (cf. Chapter IV).

This theory must be regarded as a solution theory of permeability, as it regards permeability as determined by diffusion through the plasma-membrane; it does not, however, define the chemical composition of the plasma-membrane and so might be applicable to any solution theory of permeability.

A consideration of the state of affairs postulated by the viscosity theory shows how the ultrafiltration and solution theories can be

brought into harmony, for if the protoplasmic layer determining permeability is a viscous sol or gel, the rate of penetration of a substance through the gel will, according to the formula of Einstein, Sutherland and von Smolukowski, depend on the size of the molecules as well as on the viscosity, and it is reasonable to suppose that molecular aggregates above a certain size may not be able to diffuse through the spaces between the particles of the disperse phase of the gel. Such considerations lead on to the whole question of the nature of solution into which we cannot enter here.

A number of objections to Spaeth's theory have been raised by Osterhout (1916 *a*), chiefly based on the fact that changes in viscosity of *Laminaria* thallus produced by placing this tissue in mixtures of sodium and calcium chlorides do not run parallel with changes in electrical resistance. What appears to the writer to be a very strong argument against the viscosity theory is that the changes in viscosity of a gel are scarcely great enough to account for any great differences in permeability (cf. Stiles and Adair, 1921).

The theory of antagonism advanced by Osterhout (1916 *b*) assumes, as we have seen, that the permeability is inversely proportional to the electrical resistance of a cell, and that the resistance is proportional to the quantity and the thickness of a particular substance in the protoplasm. While this theory would not be incompatible with a viscosity theory, inasmuch as the resistant substance might be a viscous one through which substances entering the cell had to diffuse, there are other possibilities. Thus, the substance to which the cell owed its resistance might be impermeable to entering substances, so that with increase in the relative amount of the impermeable substance the proportion of the protoplasm (or plasma-membrane) through which material could enter would be smaller, and hence permeability would be reduced. In such a case the question of the correctness of a sieve or solution theory is left untouched.

THE PHASE INVERSION HYPOTHESIS

To account for the supposed action of various electrolytes on cell permeability, Clowes (1916 *a*, *b*, *c*; 1917 *a*, *b*, *c*) also makes use of the colloidal character of protoplasm. As protoplasm can be regarded as an emulsoid colloidal system, there will be a continuous phase of one composition (dispersion medium) through which is dispersed at least one other phase, the particles of which are not continuous. Clowes supposes changes in permeability are brought about by the continuous and discontinuous phases changing places, as is supposed

to happen when a gelatin or agar sol sets to a gel. In this way a substance which could not diffuse through the continuous phase owing to insolubility in it might be able to diffuse through the discontinuous phase but would not be able to enter the cell because of the discontinuity of the disperse phase. With inversion of the phases, penetration would then take place at once. The hypothesis would be equally applicable to a solution or a chemical combination theory of permeability.

Clowes produces support for his theory from experiments on the effects of sodium hydroxide and calcium chloride in producing phase inversion in emulsion systems of olive oil and water, which are compared with the effects of these substances on protoplasmic permeability.

Free (1918) has subjected this hypothesis to some criticism. He points out very rightly that such a hypothesis suggests that permeability changes would be sudden, and even if it were assumed that the protoplasm (or plasma-membrane) were in a state of mobile equilibrium so that parts of it were in one state and other parts in the inverse condition, with frequent alteration in the relative quantity of the two parts which is the state of affairs Clowes appears to suggest, the balance would in all probability be very easily upset so that the whole of the protoplasm would go to one or other of the conditions.

A further weakness of the hypothesis is the absence of any direct experimental evidence in its support, for the bearing of experiments with olive oil-water emulsions on cell permeability are, as Free suggests, rather dubious, as such emulsions are not generally present in cells.

LLOYD AND FREE'S COLLOIDAL HYPOTHESIS

The hypothesis of protoplasmic permeability put forward by Free (1918) 'and earlier suggested by Lloyd (1915) bears certain points of resemblance to the two hypotheses just discussed. These writers also start out from the supposition that the protoplasm consists of an emulsoid colloidal system. It is further supposed that two (at least) of the liquid phases of protoplasm differ importantly only in the proportions of water which they contain. Changes in permeability are supposed to be due to changes in the distribution of water between the continuous phase (dispersion medium) and the discontinuous (disperse) phase. When the globules of the disperse phase are large the spaces between them will be small, and *vice-versa*, and a substance diffusing through the continuous phase will travel faster or slower according to the dimensions of the spaces. Solubility in the disperse phase alone, and not in the dispersion medium, would, of course, not permit the penetration of a substance.

A powerful argument against this hypothesis, as against the viscosity hypothesis of Spaeth, which it may indeed be regarded as including, is that actually such changes in the relative quantities of water in disperse phase and dispersion medium do not appear to produce any very great change in the rate of diffusion of substances through a colloidal system. What appears to be essentially a modification and elaboration of this hypothesis will be mentioned at the end of this chapter under the head of electrical theories.

Tröndle's Theory of Protoplasmic Irritation

It has already been noted that from data obtained by a deplasmolytic method Tröndle concluded that the rate of intake of a salt is at first independent of the concentration of the salt, and that after about the first ten minutes of exposure to the salt the rate of intake falls off according to a logarithmic relation. From this Tröndle concludes that salt intake takes place by the salt irritating the protoplasm which responds by conveying the salt into the vacuole. After the first ten minutes the protoplasm exhibits fatigue, and salt intake falls off according to Weber's law.

Tröndle (1920) considered that he had obtained confirmatory evidence of the correctness of this theory from the fact that if cells are treated with a dilute solution of a narcotic before immersion in the salt solution, the intake of the salt is retarded or even completely inhibited. The narcotic is supposed to prevent the participation of the protoplasm in the absorption process and in consequence the salt is not taken up. On the other hand, when the protoplasm is rendered inactive by preliminary treatment with dilute acid (0·01 N oxalic acid or 0·005 N hydrochloric acid) for five minutes, according to Tröndle the intake of salt is then proportional to the concentration of the salt, thus obeying Fick's law, although his figures do not appear to support this assumed relation.

In an earlier chapter reasons have been given for not accepting the conclusions of Tröndle with regard to the course of absorption. But even if the results were acceptable, the only legitimate conclusion that could be drawn from them with regard to the mechanism of salt absorption would be that the passage of salt, during the first ten minutes, did not take place by simple diffusion in a solvent, so that a solution theory of permeability to salts would be inadmissible. To conclude that a phenomenon of stimulation is in question simply because after ten minutes the intake of salt falls off with time according to

a logarithmic relation, appears to be completely unwarranted. The same result would be expected, if, after the first ten minutes, the intake of salt were a simple diffusion process governed by Fick's law. Clearly no part of Tröndle's theory can be accepted.

THE ADHESION THEORY

J. Traube, in a long series of papers (1904 *a, b, c*, 1908, 1910 *a, b*, 1911, 1913 *a, b, c*, 1919), has put forward a theory of permeability, the essential feature of which is that the capacity of a substance to diffuse into a cell depends on the extent to which it lowers the surface tension of water in contact with air. In the papers of Traube cited, the evidence for this will be found. The arguments against the adhesion theory are two, and they are fatal. In the first place the measurements of surface tension made by Traube are against air, whereas what is actually concerned is the surface tension of the solution (that in the cell wall in the case of plants) against protoplasm. There appears to be no direct relation between the surface tension of a liquid against air and its surface tension against another liquid, and there is no known way of calculating it. In the second place, as pointed out by Collander (1921), the measurements of Höber (1914, *b*) and, indeed, of Traube and Köhler (1915), show that whereas a great difference exists between acid and basic dyes in regard to their absorption by plant cells, there is no constant distinguishing difference between the two groups as regards the surface tension of solutions of them against air. Under these circumstances it does not appear worth while to discuss the theory in any detail.

The extension of Traube's theory in which the adhesion of the constituents of the protoplasm is also supposed to influence the permeability, must, as pointed out by Collander, if it is to have a definite meaning, attribute permeability either to the solvent or adsorptive properties of the protoplasm. In the first case it becomes a solution theory, in the latter an adsorption theory as described below.

CHEMICAL COMBINATION AND ADSORPTION THEORIES

A number of writers have held that some substances enter living cells by means of adsorption or chemical reactions. The substance combines chemically with, or is adsorbed by, a constituent of the protoplasm (or plasma-membrane). This disturbs the equilibrium between different cell constituents, with the result that the chemical or adsorption compound breaks down again and the substance is

released on the other side of the plasma-membrane or combines or is adsorbed by some other cell constituent according to the molecular affinities of the various constituents.

Pauli (1904) appeared to consider that salts are absorbed by their combining chemically in this way with the plasma-membrane, and the same opinion was held by Traube-Mengarini and Scala (1909) working with algæ and protozoa. The work of Szücs (1910, 1911, 1912, 1913) appears rather to suggest adsorption as responsible for the intake, and Pantanelli (1915 *a, b, c,* 1918) also lays emphasis on the importance of adsorption.

It is to be observed that this theory can be applied equally well to a cell surrounded by a plasma-membrane and to one in which the protoplasm is approximately homogeneous, and indeed Moore (1921) and his co-workers (Moore and Roaf, 1908; Moore, Roaf and Webster, 1912), who deny the existence of plasma-membranes, are supporters of this view.

Szücs (1912) appears to think a combination of the lipoid theory and adsorption theory is possible, the intake of dyes and narcotics being explained by their solubility in fatty substances, while salts enter by adsorption or chemical combination. The relation between external concentration and the position of the equilibrium attained in the absorption of salts by some plant cells can be used in support of this view, but it must be mentioned that at least some dyes appear to follow the same rule (Redfern, 1922 *b*).

ELECTRICAL THEORIES

The great difference between acid and basic dyes in regard to their penetration into cells, and the assumed influence of acids and alkalies in influencing the intake of these dyes, has suggested the possibility that the electric charge on the plasma-membrane may be of importance in determining whether particular ions are absorbed. The theories propounded (*e.g.* Girard, 1914 *a,* 1919 *a, b*) are at present mostly vague, and the foundation of fact on which they rest insecure (cf. Collander, 1921) so that a discussion of such theories in the present state of our knowledge would be scarcely profitable. It is not at all clear, moreover, whether such theories in the end do not resolve themselves into special cases of the adsorption theory of permeability.

A theory of cell permeability combining an electrical theory with a colloidal theory closely resembling that of Lloyd and Free, has recently been propounded by Miss Haynes (1921), who assumes that

the plasma-membrane is a gel of which the more solid phase consists principally of amphoteric emulsoid colloids and the more liquid phase of a buffer mixture. At the iso-electric point the continuous phase will be in a state of minimum hydration and so will occupy a minimum volume, and also will be without charge. It will in consequence be most permeable in this condition. Above the iso-electric point the membrane will possess a positive charge and so will repel kations, while below the iso-electric point the membrane will have a negative charge and will therefore repel anions. Only in the immediate neighbourhood of the iso-electric will such a membrane be permeable to ions. Modification of permeability, brought about by the addition of various substances, will, on this theory, be due to changes produced in the reaction of the buffer mixture of the more liquid phase of the protoplasmic membrane, and the phenomena of antagonism can be similarly explained.

CHAPTER XV

CONCLUDING REMARKS

IN this review of our present knowledge of the permeability of plant cells and related phenomena, I have attempted to bring together and correlate the work done by many different workers on different lines and by the use of different methods. Anyone who takes the trouble to read the literature of the subject can scarcely fail to be impressed by the isolation in thought of the majority of workers in this field. That this neglect by the individual investigator of the work of others in the same subject is not a satisfactory condition of affairs a single example will suffice to make clear. It had become evident from the work of Nathansohn and Meurer that it is possible that the two ions of a salt are not absorbed in equivalent quantities by plant cells, and that the position of the equilibrium attained in the absorption of these ions is not one of equality of concentration outside and inside the cell. If these possibilities are facts, as they have now been shown to be, then plasmolytic methods of measuring salt intake, in which the rise of osmotic concentration of the cell sap is taken as a measure of the quantity of salt absorbed, cannot give results that are beyond question. Nevertheless, during the last five years, the results of quite a number of investigations with the use of plasmolytic methods have been recorded without the authors questioning in the slightest the soundness of the method or of their conclusions. Examples of this kind can be multiplied without difficulty.

It is hoped that the correlation of work along many different lines attempted in this account of permeability will make it easier for the student to grasp the present position of our' knowledge, and also make it easier for the investigator to avoid pursuing a line of attack which acquaintance with other work in the same field would show him could not further the advance of knowledge satisfactorily.

From the review attempted in the preceding chapters it should be very evident how imperfect at present is our knowledge of the permeability of plant cells, and, moreover, how very doubtful is the significance of much of the experimental data on which our knowledge rests. It cannot then be surprising if a review of the current theories ·of cell permeability should lead to the conclusion that overwhelming

evidence in favour of any one of them is not forthcoming. To this conclusion we are certainly led, and, indeed, it would seem rather unlikely in any case that all substances should enter or be excluded from the cell on account of the same mechanism, and ultrafiltration, solubility in lipoid substances and other constituents of the protoplasm, adsorption and other surface effects, and chemical combination may all play a part in determining whether any particular substance is absorbed or not by the living cell. One point to be noticed in particular is that with all these theories of cell permeability the influence of the cell wall is usually neglected. Yet it seems to me, especially in view of the work on semi-permeable cell walls dealt with in an earlier chapter, and that of Hansteen-Cranner on the constitution of the cell wall, that this neglect is not wholly justifiable, and that to assume that the cell wall acts in no other way than as a dead porous envelope is scarcely in accord with the facts.

In conclusion it should be emphasized how overloaded the whole subject of permeability is with theories, and with observations based on the assumption of the correctness of unproved theories. For this reason many of the conclusions drawn from their observations by various investigators are valueless. Not even the membrane theory of the cell, and the simple view of the plant cell as an osmotic cell surrounded by an elastic envelope, are really proved, although the latter, within limits, has formed a good working hypothesis in regard to the water relations of the cell. It clearly breaks down when used to interpret the relation of living cells to dissolved substances. The correctness of the theory of a semi-permeable membrane surrounding the protoplasm is also dubious (cf. Davidson, 1916).

While the propounding of theories will continue to satisfy the minds of some, yet it cannot be too strongly emphasized that what are wanted to lay the foundations of a proper understanding of the phenomena of permeability in plants are facts, and particularly quantitative data. When these are abundant where they are now scanty we may be able to formulate more definitely the laws governing the interchange of substances between the cell and its surroundings, and so be in a much better position for understanding not merely the mechanism of cell permeability, but also the life of the plant as a whole.

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OF THE CAMBRIDGE BOTANY SCHOOL

THE NEW PHYTOLOGIST was founded in 1902 to afford a medium for the publication of original papers, critical articles and reviews, summaries of recent advances in botanical knowledge, and occasional notes and correspondence on botanical topics. Preference is given to those contributions dealing with matters of general interest to botanists and to those branches of science undergoing rapid current development. The journal is well illustrated, and is issued each year in five numbers. The minimum size of the annual volume is 240 pages 8vo. Annual subscription price, as from the beginning of 1921, £1. 5s. post free. Separate numbers 7s. 6d. each.